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PLUM INVESTIGATIONS
I. WINTER INJURY TO PLUM DURING DORMANCY[1]

M. J. Dorsey and P. D. Strausbaugh

(with plates x, xi)

In the breeding work with plums at the Minnesota Experiment Station, a large number of crosses have been made in which the parents varied greatly as to hardiness. Since in Minnesota in the selection of new varieties so much emphasis must be placed upon hardiness, it became necessary to devise some method of classifying parents and progeny on the basis of their ability to withstand low winter temperatures. At first it was thought that artificial freezing tests would be the best way of making such a test, but when a careful study was made of winter injury, even during relatively mild winters like those of 1915–16 and 1916–17, this method of attack was abandoned with the large number of seedlings, and reserved for use in further testing the relative hardiness of the few selections showing greatest commercial promise. Following these two seasons, the test winter of 1917–18 drew the line so sharply between the hardy and semi-hardy or tender forms that there was little difficulty in making an adequate classification, since preliminary observations had shown (1) that there were marked differences in the degree of injury to various parts of the tree, and (2) that different seedlings varied greatly in hardiness. Accordingly, a

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detailed study of winter injury was undertaken, with the object of devising a method of classifying hybrids and parents grown without protection, on the basis of the maximum injury to the tenderest tissues.

This paper is an attempt to set forth in some detail the points of injury in the plum, to show that such a plan is workable. It will be seen that winter injury has many phases, especially when the structures and growth changes involved are taken into consideration, but in this report special attention will be given to the injury occurring in fruit and flower buds, also in bark and wood during the period of dormancy.

The material used consisted primarily of seedlings of known parentage and certain of the hardier standard varieties. In either case the trees were growing under clean cultivation in a rich silt loam, without cover crops. Seven species entered into the parentage of the hybrids used, namely, *Prunus americana*, *P. nigra*, *P. Besseyi*, *P. triflora*, *P. Simoni*, *P. Pissardi*, and *P. hortulana*.

Morphological and microchemical features of fruit and flower buds

TERMS DEFINED.—There has been some confusion of the terms used in referring to the dormant fruit buds, and to the flower buds which burst from them in the spring. In order to avoid confusion in this connection, the terms fruit bud and flower bud will be carefully defined. Fruit bud is used by horticulturists generally in speaking of the dormant winter bud which is formed in late summer or early fall. These are readily distinguished from the leaf buds by differences in size, shape, and particularly in position, and are quite conspicuous after the leaves have fallen. In contrast with fruit bud, flower bud is used to refer to the developing floral structures (one to five in number) which are inclosed by the scales of the fruit bud. While the development of the fruit bud and that of the early stages of the flower bud progresses *pari passu* during the summer months, therefore, the fruit bud serves its purpose during the period of dormancy, and is superseded gradually by the flower buds upon the resumption of growth in the spring. The fruit bud is sometimes referred to in horticultural literature as the dormant bud, the winter bud, and blossom bud, or even the flower

bud. Thus the term as used by fruit growers distinguishes between this type of bud and leaf bud, and while this may imply a faulty inference with reference to fruit production, since only the flower buds initiate fruit development, the introduction of another term could not change nor clarify the popular usage. For the sake of convenience, therefore, these terms will be used as defined, because they serve to distinguish clearly between the structures involved.

FRUIT BUD.—In the plum the fruit buds are borne on short lateral and on terminal growths. The lateral growths may be thorns, branches, or spurs, and may arise from two, three, or four-year-old wood. Fruit buds vary much in shape and size, some being short and rounded at the point, as in Surprise, others being long and sharply pointed, as in *P. americana*. The overlapping scales of the fruit bud form a covering five to nine layers thick about the flower buds (fig. 1). The short, thick basal scales constitute the first covering in the initial stages of growth; later these are pushed aside by the new scales as they form within. As a result of this method of growth, the longer inner scales just inside of the basal scales are exposed only at the upper part or extreme tip, while those still farther in are completely covered. As the peripheral scales attain their full size, the exposed portions gradually die, assuming a brownish color. During winter these form a covering over the inner, succulent scales. At the apex of the bud the scales are crinkled and overlap so as to form a completely inclosed chamber containing the flower buds. This covering is made more secure by the presence of hairs on the margin and inner surfaces of the innermost scales. These hairs are outgrowths of the epidermal cells, and are characterized by a small central lumen and very thick, lignified walls.

During dormancy there is considerable space about the rudimentary flower buds as they are inclosed by the scales of the fruit bud (fig. 1). This space in some varieties is large enough to accommodate considerable enlargement in the flower buds (three to five times the dormant size) before the scales are broken. This is particularly true in some of the *P. triflora* and *P. americana* varieties, while in others with short obtuse fruit buds, as Surprise, the space is very much reduced. GOFF (7) has made reference to this feature in the cherry, and found that it varied with the number

of flower buds inclosed. During the warmer days of early spring the flower buds increase in size, and development proceeds in the anthers and pistils without any noticeable change in the exterior of the fruit buds. In the spreading apart and opening of the fruit bud scales resulting from the growth and emerging of the flower buds, there is evident a considerable adherence of these scales, due to their overlapping tips and entangled hairs. Elongation of the inner scales by reason of growth in their succulent bases extends the period of protection for the young growing structures inclosed within the bud. Soon after the flower buds break through the fruit bud in early spring the inner scales abscise at the point of attachment. The basal scales, however, persist even until the fruit is ripe. In the leaflike scales nearest the flower buds there is greater persistence than in the outer ones. Stomata are present in the fruit bud scales, excepting the outer ones which are entirely dead, but it should be stated that these were not examined in the early stages of growth. Thus it will be seen that the flower buds are protected during the early stages of growth by the fruit bud scales, but that after reaching a certain size they are liberated from them by growth and by the abscission of the scales.

Marked morphological differences occur in the inner scales of the fruit bud. There is a complete transitional series between scales and leaves on the one hand, and between scales and bracts on the other hand. The leaflike scales contain chlorophyll at the tip even before the bud is broken. These elongate most as the bud opens with spring growth, and in some species, as *P. Besseyi*, they have the form of primitive leaves. The leaflike structures, however, while borne in the position of scales, drop as late as a month or so after the outer scales.

The theory has been advanced that the insulation of the scales maintains the temperature within the fruit bud higher than that of the surrounding air. WIEGAND (34) showed such a theory to be untenable by inserting a thermometer bulb in the bud and noting that there was practically no difference between the rapidity of the fall of mercury in this bulb and that of a naked bulb. While the accuracy of this technique may be questioned, he concluded that the scales formed no insulation, but that they protected the flower buds against excessive loss of moisture through evaporation. On

the other hand, CHANDLER (2) removed the scales from peach buds in the dormant state and subjected them to artificial freezing. He found that the buds with scales removed "had very uniformly a smaller percentage killed than those still protected by the bud scales." As a result of these experiments he reemphasized WIEGAND'S conclusion concerning the protective value of the bud scales.

Microchemical studies show some interesting features of the scales of the fruit bud. The outer walls of the epidermal cells are strongly cutinized, the degree of cutinization being relatively much greater in the layer covering the outer surface of the scale. The cell walls in the browned, dead portion of the scale show some traces of lignin, and this is most marked in the region lying adjacent to the living tissue at the base of the scale (HEINICKE 12). The epidermal cells covering the living portion of the scales and two or three layers of cells lying immediately beneath the epidermis contain a considerable amount of fat, the globules being most numerous in the outermost cells, and decreasing regularly in the succeeding layers, none at all occurring in the central cells of the scales. The dead cells in the browned portion of the scales contain practically no storage material of any kind. In addition to the fat already mentioned, the living cells contain dextrin, which is the only carbohydrate stored in the bud. There is no starch present, and only slight traces of dextrose and sucrose, the latter being most marked during the early stages of growth and development. Calcium oxalate crystals are very numerous in the living tissue of all the scales, but are most abundant in the inner scales. The evolution of O_2 from the cells of the scales when a hydrogen peroxide solution is added to sections of the bud indicates the presence of catalase, and the purple color resulting when the sections are treated with benzidine shows the presence of peroxidase. All tests employed failed to demonstrate the presence of oxidase. These cells are acid in reaction, the P_H value as ascertained by CLARK and LUB'S H-ion indicators being approximately 4.6. Of the mineral substances, calcium, magnesium, and phosphorus, both organic and inorganic, are present in considerable amount; potassium also occurs in very small quantities. No nitrates can be detected at any stage in the cells of the scales or any other tissue of the buds. The biuret test indicates that there is only a very small amount of

protein in the bud scales, and that for the most part this occurs in the younger scales adjacent to the flower buds.

FLOWER BUD.—The degree of development reached by the flower bud before winter in the northern states has been stated in some detail by GOFF (6), DORSEY (4), and others. The general proportions of the parts of the fruit bud at dormancy are shown in fig. 1. It will be noted that the flower buds have reached a stage of development in which pistils, stamens, petals, and sepals are readily distinguished. The anthers reach the archesporial stage, and the pistil is present as a folded outgrowth, slightly enlarged at the base, but with no trace of ovules. Growth is resumed in early spring in all parts of the flower buds, and the normal flower may be said to terminate one phase of the cycle, since other processes condition further development.

In the plum the structure on which the flower buds are borne may be regarded as a foreshortened peduncle, in contrast with the condition found in *P. pennsylvanica*, where the flowers terminate a short peduncle, or in *P. virginiana* where the inflorescence is a raceme with a more or less leafy base. That this is the probable morphological status of the peduncle in the plum is further shown by the manner of abscission (figs. 14–16) after the flowers have fallen, and also by the fact that occasionally instances are found in which there is an elongation of the peduncle comparable with that found in *P. virginiana* (fig. 7). In each of these species the flowers have an abscission layer at the base of the pedicel, and the peduncle, whether foreshortened or not, has a single basal abscission layer. The bracts, as previously noted, borne at the base of the pedicel, are in the position expected if they were to persist after the foreshortening of the peduncle. For want of a briefer term than foreshortened peduncle, this structure will be referred to hereafter as peduncle. "Cluster base" has been used (KRAUS and RALSTON 15), but this term does not have the morphological significance, in reference to the plum, that is implied in the term peduncle.

The scales are borne on the lateral surface of the peduncle, and the lower limit is clearly defined by the abscission layer. The pith extends up into the peduncle as far as the meristematic tissue upon which the flower buds are borne (fig. 1). The term pith core has been given to this area of pith in the fruit bud or peduncle. It

is bordered by the conductive strands and is subtended at the base of the bud by the line of abscission. While the pith extends farther down into the node, attention has been given to it as limited above because it has been found to be a very sensitive index to hardiness, being browned in many instances when no other part of the fruit or flower buds was injured. On this account the pith core and peduncle will be mentioned separately in the discussion on browning or killing.

Microchemical tests show some differences between the chemical substances found in the rudimentary flower buds and those in the scales of the fruit bud. There is no storage of fat in the floral rudiments. There is, however, a very small amount of carbohydrate material in the form of dextrin. Some calcium oxalate crystals occur in the young sepals, but they are absent from all other parts of the flower bud. Also the acid reaction is not so marked in the sepals as in the fruit bud scales, the P_H value being 5 or slightly above 5. Again, compared with the scales the enzyme activity as indicated by the peroxidase and catalase tests is not so marked. The outstanding feature in the chemical composition of these tissues as contrasted with others of the fruit bud is the high protein content of the cells. Throughout the whole period of floral development the biuret reaction imparts a very high color to these tissues. A difference in this color reaction as noted for the buds of semi-hardy varieties may prove to be of considerable significance in explaining the greater resistance of so-called hardy buds.

The sepals of the rudimentary flowers become green before there is any noticeable spread of the bud scales, and the epidermis of these structures is well provided with functional stomata. On the lateral margins of the sepals there is a marked development of epidermal hairs, and in many instances these were observed to be replaced by glandular bodies made up of large secreting cells containing numerous yellowish droplets, the nature of which was not ascertained; but the protoplasts themselves are very rich in protein material and the droplets are undoubtedly secretions, for in several cases they were observed exuding from or adhering to the cell wall.

From the foregoing it will be seen that a sharp distinction can be made between the fruit buds and the flower buds. Microchemical tests also indicate significant differences in the functional activity

of the structures involved in each. The fruit bud scales protect the flower buds during dormancy, but do not function further. The complexity of the fruit bud should be kept in mind in view of the localization of the injury during winter.

Bud injury

TYPES OF KILLING.—At first thought it may appear that the injury which occurs to fruit buds during winter is so variable that it cannot be classified. Upon closer study, however, it is found that dormant fruit buds can be grouped into four well defined types or classes according to the degree of injury, as follows: (1) No injury, including all of the hardy varieties in which there is no injury even during the most severe winters. (2) Injury in which a part of the flower buds are killed but in which the bud scales and the peduncle are seldom injured. The pith core in this group may or may not be browned. (3) The type of injury in the tender forms in which all of the flower buds are killed, but in which the scales and the peduncle are injured but not killed. Under this type of injury may be included those varieties in which a few buds are not killed by mild winters. (4)This type includes those tender forms in which the entire fruit bud, including scales, flower buds, peduncle, and pith core, is killed outright.

Types two and three are less sharply defined than one and four, and semi-hardy varieties falling in group two in ordinary winters may be classified in group three after the test winters. Groups three and four appear quite distinct, because in those varieties in which all or practically all of the flower buds are killed during the usual winters, the fruit bud scales are not killed by the severer winters. The bud scales, being more closely related morphologically to leaves, appear to resemble the leaf buds in hardiness. The peduncle in groups two and three is generally not injured. In some cases, however, it is slightly browned, and sometimes the browning extends down into it from the base of the flower buds, when they are killed. On the other hand, browning in the pith core is a conspicuous feature of groups two and three.

As noted previously, the pith core was found to be so sensitive to winter conditions that injury in it was followed in detail. In

some varieties browning was restricted to a thin line bordering the meristematic tissue of the peduncle (fig. 2), while in others it occurred farther down and was much more extensive. It was not uncommon to find this area black and more or less broken down where injury was severe, and cracks or breaks in it were frequently noted (fig. 4). During milder winters browning was found in the pith core in only a part of the fruit buds, but during the severer winters it was quite general in all of them. In a few hybrids the flower buds were found to be killed, but the pith core remained uninjured. In general, however, there was a close correlation between injury to the flower buds and to the pith core. Some differences were noted between the degree of browning in the pith core of the fruit bud and of the leaf bud. In one seedling, however, a cross between Wohonka×Howards Yellow, the pith core in the leaf bud was browned while in the fruit bud it was not. When browning in the pith core has once taken place, the color remains and can readily be detected, even when the fruit is ripe. Elongation in the peduncle leaves that portion of the pith core formed before dormancy behind, and with growth in the spring new pith is laid down above the old portion, which differs decidedly from the latter in color as well as in the absence of browning.

Microchemical tests show that the pith core is essentially a storage region, and that in some respects the cells resemble those found in the endosperm of seeds. The cells are large, more or less spherical, with very thick pitted walls. The middle lamella is strongly developed, and gives the reaction of pectic material with ruthenium red. The chief thickening consists of hemicellulose, which is deposited upon the primary cell wall in large quantities. For the most part these cells are packed with storage materials, consisting principally of protein substances, fats, and dextrin. Starch was not found in the cells of the pith core at any stage of the bud's development. Quite early in the dormant period there is a decrease in the amount of dextrin, and at the same time there is an increase in the amount of fat; this change is most pronounced in the hardy buds of Assiniboine (*P. nigra*). The pith core cells of the latter species contain numerous small, colorless, spherical bodies, particularly abundant in the region adjacent to the con-

ductive strands. Microchemical tests indicate that these bodies are aleurone grains, although such a statement is made with reserve because heretofore aleurone has been found to occur only in the endosperm of seeds (TUNMANN **32**). Inasmuch, however, as the tests employed gave reactions suggesting that these are aleurone bodies, coupled with the fact that the pith core consists of storage tissue the cells of which resemble those of endosperm, it seems very probable that aleurone may be stored in dormant buds quite as well as in the endosperm of seeds. These bodies were not found in the buds of the semi-hardy forms, a fact which indicates the probable existence of another factor in the complex recognized in the term hardiness.

The P_H value of the pith core cells is approximately 4.6. The enzyme activity, as indicated by the presence of peroxidase and catalase is more marked in this tissue than in any other portion of the bud. Calcium oxalate crystals are distributed throughout this region, but are much more numerous in the cortical tissue surrounding the conductive strands. Calcium, magnesium, potassium in small quantities, traces of iron, and phosphorus are the mineral substances found in the pith core. The cells are especially rich in organic phosphorus.

When browning was first studied in the pith core, during the early spring of 1916, an attempt was made to correlate browning with the dropping of flowers which occurs so extensively in the plum, but upon further study (DORSEY **4**) this dropping was assigned to other causes. The failure of flower buds to develop, therefore, appears to be due to direct injury to them rather than to a secondary influence coming from injury in the pith core. The conducting strands bordering the pith appear to function normally in the presence of severe browning, and no instances were found in which growth in the flower buds appeared to be arrested as a result of injury to the pith core.

The greater hardiness of the leaf buds as contrasted with fruit buds was striking. In a number of tender or semi-hardy forms such as *Amygdalus Davidiana*, *Prunus domestica* var. Shippers Pride, *P. triflora* var. Burbank, and in certain hybrids between *P. triflora* and *P. Besseyi* the fruit buds were all killed, but the leaf buds made the

usual growth, excepting in those cases where the twigs were killed back. So much difference in the hardiness between leaf buds and fruit buds indicates a localization of those factors which make for hardiness not heretofore suspected. It yet remains to be seen what influence winter injury has upon development in the fruit buds after growth starts in the spring.

Dropping of winter killed buds.—The line between flower buds which have been killed during winter and those which have not been injured is relatively sharp. While this material was not examined extensively for slight or localized injury in the flower buds, it appeared that during the severer winters when a flower bud was injured, in the great majority of cases it was killed. It would be reasonable to expect gradations in injury, however, although but few buds can be found in the plum which have started growth and then died, except where killing conditions have prevailed late in the season. In hardy forms where none of the flower buds are killed, as in group one, growth proceeds in a normal manner in all of the parts of the fruit bud. Likewise, when all of the flower buds in a fruit bud except one are killed (group two), development in the peduncle and abscission in the fruit bud scales take place as if no injury had occurred. When all of the flower buds are killed (group three), or the entire bud is killed (group four), the usual sequence of events is greatly disturbed. In these cases the fruit bud never opens, and abscission takes place at the base of the peduncle (figs. 1, 2). Instances are common in some varieties (Okaya) where three fruit buds borne at a node on the terminal shoots have a common abscission point (fig. 6). Winter injury, therefore, as in groups three and four, does not result in immediate abscission, and it is not until spring growth takes place in the twigs that the fruit buds fall.

The dropping of winter killed fruit buds reaches its height in most varieties about blooming time. A number of agencies hasten abscission. The most active appear to be wind, rain, drying, and crowding from growth in the spur or twig, or from the enlargement of other fruit buds in which all of the flower buds have not been killed. Abscission, however, does not take place if the fruit spur or limb is killed. After a bud drops the exposed area soon dries and

contracts, and a protective layer of dead cells forms over the exposed part.

ABSCISSION POINTS.—It will be seen from the foregoing that winter killing may affect development directly in the fruit bud as a whole, in the scales only, or in the flower buds. There are so many structures involved in the life cycle of fruit and flower buds, that it is interesting to note that abscission following winter injury fits the morphological status given the peduncle. The fruit buds in groups three and four abscise at the same point as does the peduncle later on if none of the flowers arising from a fruit bud set fruit. The scales have definite basal abscission points, which show clearly on the elongated peduncle (fig. 5), and while they do not fall in group three because of the adherence occasioned by overlapping, abscission layers are formed, as is shown by figs. 1, 2, and 5. The formation of the abscission layer in the fruit bud scales then appears to be independent of the formation of the abscission layer at the base of the peduncle, since in the latter it may be formed at four distinctly different times: (1) when all of the flower buds are killed; (2) when the flowers develop but do not set fruit; (3) when the fruit sets but drops at the third or June drop, and (4) at maturity after the fruit is picked. Abscission may be prevented in either the fruit bud, peduncle, or fruit stem by drying in the region of the abscission layer before it is completely formed. It should be noted here that the style, pistil, petal, and calyx tube each have separate abscission points, and that the stamens do not abscise, but fall with the calyx tube.

FORMATION OF ICE WITHIN BUD.—Along with the study of the localization and extent of injury, an investigation was made of the condition in the dormant fruit bud when it was frozen during winter. Twigs were collected from the trees during winter at times when the temperature was low, and the buds were studied without being thawed with a binocular microscope on the window sill, outside of the laboratory. In this way the temperature of the buds was practically that of the air throughout the examination.

When the fruit buds were dissected at temperatures as low as $-14°$ F., the spaces between the scales were found to be packed with ice crystals. This condition in frozen buds had previously

been observed and reported by WIEGAND (**34, 35, 36**). There was evidence of some ice formation within the pith core, but in no other portion of the peduncle or within the tissue of the bud scales. The space occupied by the ice crystals between the successive scales is considerably larger than it is normally, so that the bud as a whole appears swollen and distended. This difference in size and general appearance is easily detected with the unaided eye, and is very marked when observed under the binocular.

One of the surprising features of the ice formation in the fruit bud is the absence of crystals in the bud space inclosed by the scales, above and around the flower buds. This phenomenon is an important item in hardiness, since it does not bring the tender and delicate flower buds in direct contact with ice crystals, which would result in injury either directly or by crowding. It may be, however, that crystals are formed on the surface of the flower buds and on the inner surface of the scales at lower temperatures than those under which these observations were made. No attempt was made at this time to determine the movement of the water when the ice held between the scales thawed, but WIEGAND (**34**) held that it was again taken up by the scales.

When these observations were extended to still lower temperatures, $-21°$ F., the flower buds were found to be soft and pliable, and free from ice crystals. When they were teased apart with scalpel or needle, the cell contents appeared to be more viscous and somewhat darker than at higher temperatures. The difference in reaction to low temperatures between the fruit bud scales and the flower buds is interesting, and indicates differences between them in keeping with the morphological status of each. It should be pointed out here that the temperatures at which these observations were made were not low enough to kill any of the varieties of *P. americana* under observation, and also that sections of the wood upon which the buds were borne showed an abundance of ice crystals.

The extent of ice formation in the bud at temperatures lower than $-21°$ F. was not determined, but it would appear that below a certain critical temperature for each variety, a point is finally reached at which a sufficient amount of water is withdrawn to cause

permanent injury to the protoplasts. If the imbibitional forces within the protoplasm are strong enough to retain the water against the dehydrating effect of freezing, the critical temperature is much lower and the variety is classed as hardy. If the temperature does not fall low enough to cause permanent injury, it is probable that as the ice in the bud melts the water is reabsorbed, providing the thawing does not take place too rapidly. On the other hand, when the cell is permanently injured the permeability of the plasma membrane is so changed that the water is not taken up by the protoplast, and the tissue soon presents a dark, water-soaked appearance. The flower buds, however, may undergo a considerable degree of supercooling, the extent depending upon the hardiness of the variety in question. It will be interesting now to examine the killed flower buds in more detail.

CYTOLOGICAL CHANGES IN KILLED CELLS.—An examination of cytological preparations of winter killed flower buds shows that marked changes occur in the cell organization as a result of freezing to death of the tissue. In sections of normal living cells the plasma membrane, where slightly shrunken away from the cell wall, appears as a clear, distinct line staining uniformly throughout with either Heidenhain's hematoxylin or the triple stain. The nuclear membrane is also clear and well defined, and the staining of the chromatin meshwork shows the normal organization of this material as found in resting nuclei in general. On the other hand, in sections of killed cells both the plasma membrane and the nuclear membrane have disappeared as distinct, limiting membranes. The peripheral regions of the cells are now delimited by an irregular margin that stains very heavily. The general appearance suggests that the semi-permeability of the plasma membrane has been lost, and that the stain is absorbed in large quantities, forming a dense, irregular blotch.

The outline of the nucleus is likewise lost, and there is a dense smutch of stain in this part of the cell. In many of the cells every detail of the nuclear organization is completely obliterated. Where safranin is employed as the nuclear stain the area in which the stain is absorbed is often much larger than that occupied by the nucleus of a normal cell. Safranin, being a basic stain, reacts with the

nucleic acid, and the inference is that the chromatin has been disorganized, with the result that an acid radical is released. The nuclear membrane is broken down and the acid diffuses outward into the protoplast, so that the stain reacting with it forms the dense, irregular smutch mentioned. These changes are best observed in the flower buds, in the cells of the calyx tube, or the pistil. The cell walls show no indication of rupture or breaking apart as a result of freezing; nor was there any evidence that ice crystals had formed in the tissues of the flower buds where the cells lie very close together and in which there are no intercellular spaces present.

It is apparent, therefore, that killing in the embryonic flower buds brings about fundamental changes in the cell organization, particularly in the nucleus. From this evidence killing may be looked upon as a phenomenon of the cell, and the initial causes which bring it about ultimately will have to be studied from this point of view.

Bark and wood injury

Varying degrees of browning are found during a given winter in the bark and wood of the plum, especially when the different varieties and species are concerned. While the degree of browning in the bark and wood are closely correlated, the intensity of browning in the bark is in general less than in the wood. This difference is significant in view of the fact that both must of necessity undergo similar conditions within a relatively narrow range.

The relation of the tree to its environment during the dormant period is extremely complex. Such considerations as size, angle and direction of twig or branch, and twig color (WHITTEN **33**), all have a bearing upon temperature changes in the tissue. The work of MIX (**20a**) with the apple, HARVEY and DORSEY (**11**) with trunk temperatures, and others, amply demonstrates that the temperature changes constantly both as to extent and rapidity in the bark and wood. These fluctuations result in localization of injury, such as sun scald, in addition to the more general injury which is conditioned by the inherent resistance of varieties and species. It is only when the cambium is injured or killed that "killing back" or "die back" takes place. In view of the greater hardiness of

the cambium, therefore, it will be interesting to consider injury in the bark and wood in more detail.

CHANGES IN BARK INDUCED BY LOW TEMPERATURES.—The outer portion of the bark of the older twigs consists of several layers of suberized cells, which make it quite impervious to water except through the lenticels. The cortical cells underneath the cork contain considerable chlorophyll and an abundance of storage material, chiefly fat. The ray cells of the bark are packed with fat, and fat containing cells are scattered all through the cortical tissue. No starch is found in the bark. All of this tissue is rich in peroxidase and catalase.

In cases where the injury is slight the browning that results is confined to cortical cells containing chlorophyll and storage products other than fat. When sections of slightly injured twigs were treated with benzidine and hydrogen peroxide, those cells which had browned did not give the purple reaction obtained in uninjured twigs. Since this color is due to the oxidation of benzidine by peroxidase, it appears that some change is wrought in the enzyme activity when bark is injured by low temperatures. The enzymes may be used up or inhibited in some way in the reaction which manifests itself in the browning. In cases of extreme injury the browning extends to all the tissues. Except in those instances where the twig is killed outright, the cambium appears to be uninjured, and resumes its activity with the return of growth conditions.

Bark characters vary widely in different species of the plum, both as to internal structure and external features such as color, degree of smoothness, and lenticel development. The hardier forms in general are characterized by a smoother, tighter bark than that of the less hardy forms. The bark of Assiniboine, one of the hardiest of the plums, is very smooth, possesses a high luster, and is dark gray. It has a very small number of lencitels per unit of surface as compared with some of the less hardy varieties, there being, for example, three to six times as many lenticels in Stella as in Assiniboine on the same extent of twig surface (STRAUSBAUGH 31). All of these factors may be of importance in an indirect way in determining the greater resistance to low temperatures manifested by hardy forms such as Assiniboine.

CHANGES IN WOOD INDUCED BY LOW TEMPERATURES.—Browning of the wood, resulting from low temperatures, is due in part at least to a condensation of storage materials, which thereby apparently are transformed into gums and tannins. During the winter months the ray cells of the normal wood contain large quantities of fat and very little starch. As the warmer weather of spring approaches this situation is reversed, and these same ray cells become packed with starch, very little fat being present. When sections of the wood in which browning has occurred are examined, the vessels are seen to be partially occluded by yellowish brown masses. Some vessels are particularly free from such occlusions, but none is completely filled. These occlusions are most pronounced in the older vessels of the browned wood ring, so that concentric rings of deeper color alternating with rings of a lighter shade appear in the transverse section of a branch that has been affected by the subminimal temperatures of a number of successive winters. As already stated, unless the tree is killed outright the cambium is not injured, and will give rise to new wood during the subsequent growing season.

When sections of the browned wood are treated with hydrochloric acid and 1 per cent osmic acid, most of the yellowish brown, occluding masses become green, a reaction which indicates the presence of tannin. Others of these masses react with tests for various substances found in gums. For example, the Fluckiger test with prolonged heating reveals the high dextrin content of many of these occlusions. Also, when sections are treated with phloroglucin and concentrated hydrochloric acid, and heated 10–15 minutes, many of these masses develop a beautiful cherry-red color, a reaction showing the presence of pentoses. Hence it is fairly safe to state that these colored occlusions for the most part consist of a mixture of gums and tannins.

The ray cells of browned wood contain very little storage material, but some of the wood lying just within the discolored ring may have the ray cells filled with starch, while the new wood formed the subsequent season adjacent to the browned wood will have ray cells stored with fat. This would seem to indicate that the presence of these injured cells in some way interferes with

the transformation and transfer of the storage materials lying in the cells of the wood of the older rings.

The intensity of browning in the wood has been used as a basis for classification, and, on account of the permanence of the browning, the effect of one season can be compared with another, especially when milder winters follow severer ones. Within the limits of the material available for this study, it may be said that in general, hardiness in wood, bark, fruit bud, and cambium are correlated, yet when the material as a whole is surveyed considerable variation is found in the relative hardiness of each.

INFLUENCE OF BUD KILLING UPON FRUITING LATERALS.—Fruiting laterals in the plum may be divided into two quite distinct groups, (1) those which bear only fruit buds, and (2) those which bear both fruit buds and leaf buds; the latter are usually borne in the terminal positions. During the severer winters when the bud killing is extreme, all of the fruit buds on many of the fruiting laterals may be killed (figs. 13–16). When this occurs, laterals without leaves become inactive immediately, although the base may remain alive for some time. If these were to set, fruit activity would continue until the fruit ripens. In either case lateral vegetative growths are seldom formed on fruiting laterals which do not have a growing point in the spring when growth starts. On the other hand, when a leaf bud or growing point is present, they (being hardier than fruit buds) are not killed except when the laterals are killed outright and the activity of the lateral is continued.

In table I data are presented to illustrate the effect winter injury may have through bud killing upon the fruiting laterals. The varieties listed here differ in hardiness. Assiniboine is seldom injured by winter, while the others grade into the semi-hardy class in Minnesota. Note the slight killing in Assiniboine when contrasted with Tonka or Abundance×Wolf no. 35. In each instance an analysis was made of the fruit bud killing on all of the fruiting laterals of a branch, the age of which is given in the table. Following Tonka through the table, it will be seen that in 1916, out of a total of seventy-nine living fruiting laterals, forty had no terminal leaf or vegetative buds and had all of the fruit buds killed. These functioned no further, whereas the nine laterals

have no terminal vegetative buds, but bearing flower buds may remain alive until the fruit ripens. Contrast the condition in this variety the following year (1917), during which the killing was less severe, and out of 183 living laterals only eleven, which had no terminal fruiting buds, had all of the fruit buds killed. It will be noted Opata has the terminal fruiting habit. The effect of winter killing on the fruiting laterals in the hardy variety, Assiniboine, is very limited when compared with Tonka. In this way it will be seen that winter killing may be a factor in hastening the suppression of laterals in the central part of the tree on the older wood.

TABLE I

EFFECT OF FRUIT OR FLOWER BUD KILLING UPON FRUITING LATERALS

Variety	Year	Age in years of branch bearing fruiting laterals (thorns, branches or spurs)	Total number of fruiting laterals alive	Fruiting laterals dead from previous season	Total number of fruiting laterals with growing point; fruit buds on these may or may not be killed	Laterals bearing living fruit buds, but no leaf buds	Laterals having no leaf buds on which all of fruit buds were killed
Abundance×Wolf no. 35	1916	5	103	93	45	15	43
Abundance×Wolf no. 35	1917	4	197	183	101	60	36
Assiniboine	1917	5	237	47	148	85	4
Burbank×Wolf no. 6	1916	4	174	23	91	66	17
Tonka	1916	3	79	15	30	9	40
Tonka	1917	3	183	27	100	72	11
Opata	1916	3	108	4	108	0	0
Opata	1917	5	174	152	174	0	0

RATE OF TEMPERATURE CHANGE AS FACTOR IN KILLING.—Different investigators have given considerable attention to the rate of temperature change as a factor in killing. PFEFFER (**26**) states that "resistant plants withstand rapid and slow cooling equally well, and it is doubtful whether a rapid fall of temperature is more injurious to plants killed by freezing than is gradual cooling." WINKLER (**37**) finds that the rapidity of cooling is an important factor in killing. He maintains that twigs can endure from four to six times as much cold if the temperature change is a gradual one. CHANDLER (**2**) reports that rapidity of cooling exerts great influence on the extent of killing, especially in the case of

winter twigs and buds. POTTER[2] reported data showing that rapid temperature fall is a very marked factor in killing.

While at the present time it is generally conceded that the rate of temperature fall may determine to a marked degree the extent of killing, no such unanimity of opinion prevails concerning the possible influence of rapid thawing on the killing of tissues. SACHS (27) contended that rate of thawing determined the amount of injury. MULLER-THURGAU (23), MOLISCH (21), and WIEGAND (34) find that rate of thawing has no influence on the extent of killing. HABERLANDT stresses the effect of sudden thawing. SALMON (28) states that "slow thawing can reduce the injury only on the assumption that death occurs after the tissue begins to thaw. The only theory so far advanced that accounts for winter killing in this way is by permanent loss of water from the protoplasm. In such cases slow thawing would permit water to be reabsorbed which if thawed rapidly would be evaporated into the air."

Some experiments were made in this connection which indicate that rapid thawing may be a factor in the injury or killing of plum tissues. Twigs of a number of varieties and of fifteen hardy seedlings of Surprise (*P. hortulana*) were collected in duplicate lots in January 1917, when the temperature was −18° F. One lot was taken into the greenhouse immediately and allowed to thaw at a temperature of 80° F. The other lot was brought into the warm greenhouse gradually by two hour intervals (from −18° to 80° F.) as follows: first, in the root cellar entrance with a temperature of −4° F., then in the root cellar where the temperature was 13° F., next in the cutting and bulb room at 35° F., and finally into a cool greenhouse and then into the warm one. This schedule was completed between 8 A.M. and 6 P.M., and was decided upon because it is probably more rapid than any temperature changes which ever occur naturally.

Browning appeared in the bark of the plum twigs brought in immediately in about two hours, the deepest browning being above the water level in the beaker. Some of the flower buds appeared brown soon after thawing, and some of the more hardy

[2] Paper read before Great Plains Section of the American Society of Horticultural Science, St. Paul, Minnesota, July 8, 1920.

did not. All of these twigs developed leaves and some flowers when allowed to remain in the greenhouse from three to four weeks. In contrast with the twigs brought in immediately, those brought in according to the schedule just described were not injured in any part. It should be stated that the twigs of none of the Surprise seedling were injured outside during the winter of 1916–17, and no records were taken in other years.

These tests were repeated in 1918 under similar conditions, and twigs of *Cornus stolonifera*, *Ulmus americana*, *Acer saccharinum*, and *Syringa vulgaris* were added to the list. The plum twigs responded in the same way, but there was no injury to any of the native species listed, even by the sudden thawing.

With the results of these experiments in mind, an attempt was made to observe the changes more closely under controlled conditions. Accordingly, four containers were packed in sawdust, each one containing a different concentration of a freezing mixture of calcium chloride and ice. The theoretical temperatures which should have been reached in each container were as follows: no. 1, −10.9° C.; no. 2, −21.5° C.; no. 3, −40.3° C.; and no. 4, −54.9° C. There was difficulty in getting the different concentrations mixed properly, so that the theoretical temperatures were not reached. Twigs of Assiniboine, Tonka, and Stella were placed in a corked test tube with a thermometer passed through the cork. This tube was then placed in a still larger tube, so that it was surrounded with a jacket of air. The larger tube was then immersed in the freezing mixture. The air jacket retarded both freezing and thawing. The temperature came to a standstill in the tubes containing the twigs and thermometer in approximately one hour. After the temperature had come to a standstill in the lowest chamber at −57° C., one twig of Assiniboine was taken out into laboratory temperatures (72° F.) immediately and the fruit bud scales removed. The flower buds were observed through the binocular microscope, and as they thawed they turned brown. It should be remembered that Assiniboine is one of the hardiest varieties on trial at the Minnesota Station. After the browning was observed in Assiniboine it was studied in the other varieties, and the time of the appearance of browning was carefully noted. Following the treat-

ment given these twigs, in which both rapid freezing and rapid thawing are involved (especially the latter), the flower buds assumed the characteristic brown color of dead buds in the surprisingly short time of two or three minutes after being removed from the temperature stated.

The appearance of the buds frozen and thawed in this way corresponds with that of flower buds killed under orchard conditions. In the plum the killing in the flower buds appears to be more general than that which occurs in other fruits, notably the cherry and peach. Even in the milder winters when the killing is slight, partial or local injury to the flower buds is relatively rare, judging from external appearances. For this reason attention has been directed toward the pith core and tissues other than the parts of the flower bud, such as the pistil or anther. In later development, however, spring freezes do affect special parts without killing the bud as a whole.

The difference of opinion concerning the effect of the rate of thawing and freezing may arise largely from the character of the observations made. If a growing test is taken as a criterion of injury instead of a browning test, different conclusions might be drawn, since the cambium may not be injured under the same conditions as the bark, wood, or buds. While the change in bringing twigs from −18° F. immediately into a warm palm house at 80° F. is much more rapid than those involved in usual weather changes, these experiments were purposely carried out in this way in order to determine whether it was possible to induce browning comparable with that observed in the orchard. It will be seen from the experiments, using the browning test as the index, that rapid thawing as well as freezing may result in injury. These two factors, however, cannot be separated in the artificial freezing tests, but it should be noted that the browning in the flower buds did not appear until the flower buds thawed.

RELATION BETWEEN DORMANCY AND WINTER INJURY.—*P. nigra* and *P. americana*, two of the hardiest species, are characterized by a long period of dormancy, while *P. triflora* has an exceedingly short rest period. Hybrids between the first named species and *P. triflora* appear to stand near midway between the parents in re-

spect to the length of the rest period. In *P. nigra* (Assiniboine) the first break in dormancy, as indicated by anthesis when twigs are placed in water in the greenhouse, occurs toward the end of January, while in some of the other varieties, such as Tonka, twigs cut as early as October 3 bloomed in a little over two weeks (STRAUSBAUGH **31**). The species noted here indicate the range in the rest period encountered in the varieties under observation.

This relationship is emphasized here because the rest period has a direct bearing upon the time of killing (HOWARD **13, 14**). While maturity also influences hardiness, it alone does not induce sufficient hardiness to prevent killing in forms otherwise classified as semi-hardy or tender. The time of the killing as indicated by the first appearance of dead flower buds was found to occur each year near midwinter.

In 1916–17, the first dead flower buds were found January 21; in 1917–18, on January 29; in 1918–19, the first week in February; and in 1919–20, the killing was so slight that the time was not ascertained. During these winters the percentage of killed flower buds did not increase as the season advanced, indicating that with plums in Minnesota there is generally a single killing period. In Minnesota, however, the time of killing cannot be determined exactly by the time of the appearance of the dead flower buds, because several weeks may elapse between the critical temperatures and thawing, when the browning first appears, but the evidence indicates that the rest period is over by the time of killing.

Other investigators have noted specific killing dates. MORSE (**22**) shows that the time of injury to apples in Maine in 1906–7 can be ascribed to a single cold period in January. MACOUN (**17**) found apple scions to be injured between December 5 and February 13. The localization of the killing period and the appearance of browning in the buds and wood upon thawing have been noted by CHANDLER (**3**), who states that in the case of the buds killing occurs on a single night, and death can be observed soon after the buds have thawed. In case of the wood, it can be killed by a single freezing of a few hours. Other instances are found in horticultural literature where killing is known to have occurred in short critical weather periods.

Discussion

It is apparent that in using the terms browning and injury it is difficult to fix definitely the meaning of each. Browning has been used here to refer to the discoloration found in the tissues of the bark, wood, or bud. This term is also used as an index of injury, and where the browning is slight the injury is considered to be slight, and likewise when the darker shades of discoloration are found injury is considered more severe. This use of browning appears to be justified in view of the fact that after an extensive survey of the plums as well as of other fruits, killing is found accompanying the darker shades of discoloration with few exceptions. On the other hand, it is evident that injury must be described or defined ultimately in terms of cell injury, although this term is used to refer to injury to tissues as well as to cells. Some of the characteristics of injured cells have been pointed out. It is difficult to state just how far impairment or disturbance in the functioning of a cell can take place without effecting some visible change. The influence upon diffusion and transportation which arises from the condensation of storage materials under the influence of low temperatures is problematical, especially in the initial and slighter stages. All of these studies were made upon mature wood and bark, and upon the flower buds during dormancy, and admittedly there is some question as to the advisability of using injury to refer to changes in meristematic tissue, to lignified vessels, to cambium, and to cortex. In the wood, browning apparently is due to changes which form non-transportable products that seem to interfere both with the mechanism of correlation and of digestion. The meaning of injury may not be as specific, therefore, as could be desired, and, like other scientific terms, it loses its distinctness with the extension of knowledge.

Much has been written concerning the probable cause of the killing of tissues at low temperatures. Reviews of the literature are given by CHANDLER (**2**), SALMON (**28, 29**), and HARVEY (**10**). DUHAMEL and BUFFON (**5**) believe that death is caused by mechanical rupturing of the cells due to the formation of ice crystals within the cell. MULLER-THURGAU (**23**), MOLISCH (**21**), and others regarded death as a result of the withdrawal of water from the cell

in the formation of ice crystals in the intercellular spaces. GORKE (8) thinks that killing is due to a precipitation of proteins occasioned by the withdrawal of water in the formation of ice crystals. MAXIMOW (20) holds that the killing effect is limited to the plasma membrane. His experiment showed that various organic and inorganic substances afforded a protective influence, provided they were not toxic and their eutectic point was not too near the freezing point. HARVEY (10) has recently reported that the protein precipitation resulting from freezing is due chiefly to the increase in H-ion concentration and an increase in salt concentration. CHANDLER (2) and OHLWEILER (25) emphasize the close relation existing between sap density and resistance to low temperatures. MATRUCHOT and MOLLIARD (18, 19) and GREELY (9) regarded the effects of freezing, desiccation, and plasmolysis as essentially the same. HARVEY agrees with this view, because these processes cause similar changes in the H-ion concentration and salt concentration.

A number of investigators now incline to the view that death of the cell is due to the withdrawal of water, the principal question being as to ultimate effects of the removal of water upon the chemical or physical structure of the protoplast and that of the plasma membrane. LIDFORSS (16), SCHAFFNIT (30), and others regard the formation of sugars at low temperatures of extreme importance in the prevention of killing, as sugar has a protective action for colloids and therefore the precipitation of proteins does not take place. WINKLER (37) has emphasized the ability of the plant to acclimatize itself to the gradual seasonal changes in temperature, and this is undoubtedly a very important factor in the ability of plants to withstand low temperatures.

Within the limits of the observations made in this investigation, there seems to be very little indication of morphological differences between hardy and semi-hardy forms that could account for differences encountered in resistance to low temperatures of the forms under investigation. Such morphological differences as do exist are more likely the result of a physiological differentiation, which in all probability is the immediate cause of the differences in resistance. BEACH and ALLEN (1) make reference to the importance of maturity as a determining factor in hardiness, and CHANDLER

(2) says that maturity is "the most important factor affecting the hardiness of plant tissue." By October 1, *P. nigra* (Assiniboine) had formed the abscission layers for the leaves which were already yellowing and beginning to fall. This was not true of any of the semi-hardy varieties, which gave no sign of such maturity until two or more weeks later. This seems to be another indication of a correlation of early maturity with hardiness, and, although at present a complete analysis cannot be made of the condition termed maturity, in it is recognized the expression of certain physiological processes which enable tissues to withstand low temperatures within the limits characteristic of the species.

As previously mentioned, there are indications of a difference in chemical composition between the cells of hardy and those of semi-hardy buds. The hardy buds appear to contain more fat, and their protein content is different from that of the semi-hardy buds. This means that there is a difference in the colloidal structures of the cells, and accordingly a difference in the imbibitional force which they manifest. From evidence reported elsewhere (31) it is shown that water loss in *P. nigra* is much slower than that in other semi-hardy varieties, and such a situation would lead us to expect just such a difference in imbibitional force as has been suggested.

It is not likely that hardiness will be accounted for by a single factor; undoubtedly many factors are operative. Different factors may be involved in different locations and with different species. Even in the same plant different tissues respond differently. Hardiness must be thought of in terms of a complex of causative factors all working together to enable the plant as an organism to resist low temperatures. Some of these factors may be best sought in the inheritance of the plant; others in the internal cell composition and structure; and still others in the environment of the plant.

Summary

1. The fruit bud is considered to be the dormant winter bud which is formed in late summer or early fall, as distinguished from the flower buds, one to five in number, inclosed by the scales of the fruit bud. While both structures are formed in part at the same

time, the sequence of development is different in each, and this distinction will contribute toward clearness in the use of terms.

2. In the inner scales of the fruit bud, a complete transitional series can be found between scales and leaves on the one hand and scales and bracts on the other. The structure on which the flower buds are borne in the plum may be regarded as a foreshortened peduncle, and therefore as constituting one extreme of a series with *P. pennsylvanica* and *P. virginiana*. The central part of the peduncle, called the pith core, is a very sensitive index to hardiness.

3. The pith core is essentially a storage region, its cells being packed with materials consisting principally of protein substances, fats, and dextrin. Starch was not found in these cells at any stage of the bud development. The P_H value of the pith core cells is approximately 4.6. Of the mineral substances, calcium, magnesium, potassium, iron, and phosphorus were found.

4. The different forms under study can be grouped into four distinct classes as to hardiness in the fruit buds: (1) the hardy forms which are not injured; (2) those in which some of the flower buds are killed but in which the fruit bud scales and the peduncle are seldom injured; (3) those in which all of the flower buds are killed (the peduncle and scales may be injured but are not killed); (4) the tender forms in which the entire fruit bud is killed.

5. When all of the flower buds are killed on fruiting laterals which do not have leaf buds, such laterals are suppressed immediately. If fruit is set, however, activity continues until harvest time. In this way winter killing may bring about early inactivity in a considerable proportion of the laterals on a tree. All of the flower buds on laterals may be killed, however, and the activity of the lateral be continued to the next season if it has a leaf bud.

6. Experiments performed with twigs during cold weather indicate that the rate of thawing may be an important factor in killing as well as the rate of freezing. Browning appeared in flower buds of Assiniboine upon thawing when artificially frozen, in two to three minutes.

7. Killing in the flower buds was found to occur near midwinter each year at a time corresponding closely to the break in the rest period.

8. When the fruit bud is frozen during the low temperatures of winter, ice forms between the scales of the fruit bud but not within the space about the flower buds.

9. Cytological studies of winter killed flower buds show that the nuclear organization of the cells is broken down, but that the cell walls show no indication of rupture or breaking apart.

10. In the bark where injury is slight the browning is confined to cortical cells containing chlorophyll. No starch was found in the bark, but the ray cells are packed with fat, and fat containing cells are scattered throughout the cortical tissue. Bark characters vary widely in the different species of plums as to internal structure and external appearance.

11. Browning in the wood is due, in part at least, to a condensation of storage materials, which apparently are thereby transformed into gums and tannins. In browned wood the vessels are partially occluded by yellowish brown masses, the occlusions being most pronounced in the older vessels of the wood, forming the characteristic, concentric appearance of transverse sections of injured limbs.

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EXPLANATION OF PLATES X, XI

PLATE X

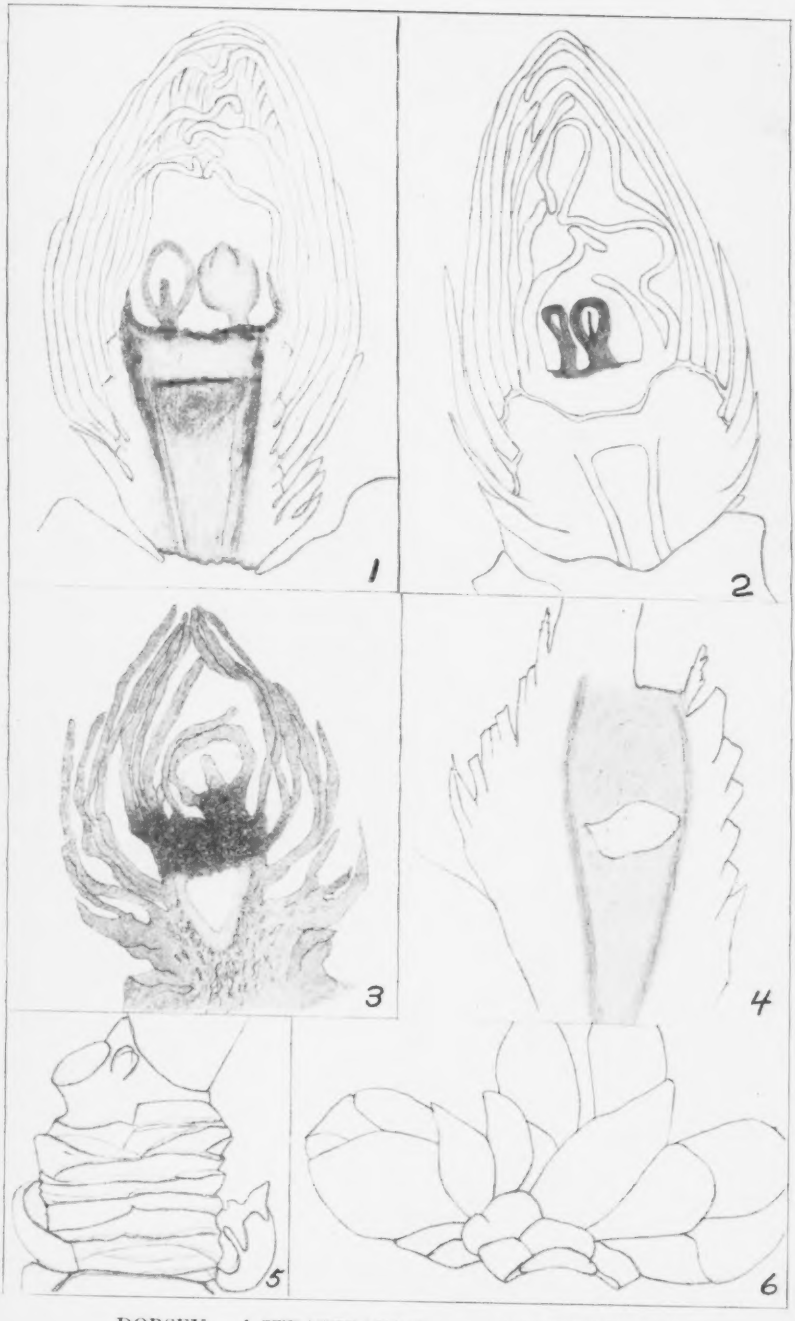
FIG. 1.—Longitudinal section of fruit bud of Red Wing in which flower buds are killed, made two weeks before bloom to show browning in pith core and space above flower buds; note hairs at tip of scales and line of abscission at base of bud.

FIG. 2.—Tonka: drawn to show line of abscission of scales of fruit bud and peduncle; dead flower buds are being cut off with scales; abscission layer not yet formed in basal scales.

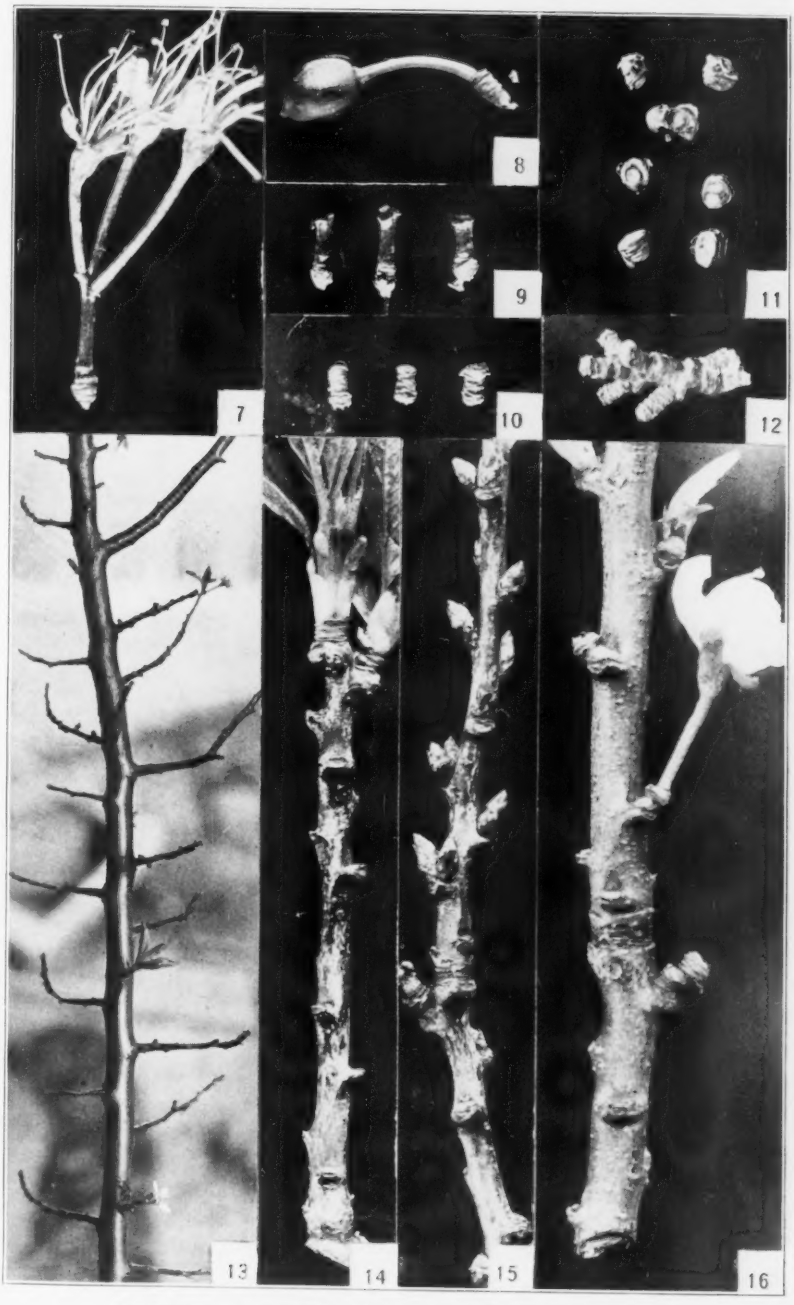
FIG. 3.—Section through dead fruit bud of *P. Simoni;* note dried shrunken appearance, cracks in peduncle, and intensity of browning; buds like these do not drop with resumption of growth in spring and can be found in many instances persisting for two or more years.

FIG. 4.—Okaya: section through peduncle showing broken area in pith core such as is frequently found during dormancy; such breaks enlarge with growth in peduncle but do not appear to affect conducting strands bordering pith core.

FIG. 5.—Seedling of Abundance×Wolf: peduncle showing scars formed by abscission of scales, thick basal scales still present; at top is shown at left



DORSEY and STRAUSBAUGH on PLUM HARDINESS



DORSEY and STRAUSBAUGH on PLUM HARDINESS

the scar of pedicel which has dropped at first drop, in center a dead flower bud; and at right the point of abscission in pedicel of the flower still persistent.

FIG. 6.—Okaya: showing common point of abscission when all flower buds are killed in fruit buds borne at a node.

PLATE XI

FIG. 7.—Late Goose (*P. americana*): three flowers of fruit bud borne on elongated peduncle similar to *P. pennsylvanica*.

FIG. 8.—General proportions of pedicel and peduncle after fruit has set.

FIG. 9.—Elongated peduncles of *P. americana*.

FIG. 10.—Normal peduncles in *P. americana*.

FIG. 11.—Short thick peduncle of Stella (*P. triflora*×*P. americana*).

FIG. 12.—*P. americana:* peduncles left on spur after all flowers had fallen at first drop.

FIG. 13.—Seedling between Abundance and Wolf (no. 35), showing influence of bud killing on fruiting laterals (table I).

FIG. 14.—Fruiting lateral of Tonka, with two growing points on which all of fruit buds have dropped after growth started in spring, and showing typical scars after winter killed buds have been cut off by abscission.

FIG. 15.—Winter killed buds on seedling between Burbank and Wolf (no. 6), before dehiscence.

FIG. 16.—Another seedling between Burbank and Wolf (no. 16), showing peduncle and dead flower buds after scales have been removed; note that one flower is left.

STUDIES IN THE GENUS BIDENS. VI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 311

EARL EDWARD SHERFF

(WITH PLATES XII–XIV)

Bidens amoena, sp. nov.—Herba erecta, verisimiliter perennis ac 5–10 dm. alta, amoena; caule glaberrimo, obtuse tetragono, demum plus minusve stramineo, parce ramoso. Folia tenuiter petiolata petiolis 1.5–4.5 cm. longis, petiolo adjecto 9–17 cm. longa, bipinnatisecta vel tripinnatisecta, lobis primariis paucis remotisque, terminali usque ad 5 cm. longo, ultimis oblongo-linearibus vel elliptico-linearibus, 0.6–3 mm. latis, integris, pilis subtenuibus non numerosis interdum ciliatis, submembranaceis, glandulis minutis multis conspersis, supra satis viridibus, infra pallidioribus, versus apicem primum sensim demum saepe subabrupte angustatis, apice ipso acutissimo mucronatis. Capitula pauca, ramos terminantia, radiata, pansa 3–5.5 cm. lata et 9–11 mm. alta, tenuiter pedunculata, pedunculis foliolis linearibus 1–4-bracteatis, ± 5 cm. longis. Involucrum basi hispidum; bracteis exterioribus 9–12, lineari-elongatis, acutissimis, 8–11 mm. longis et circ. 1 mm. latis, patentibus vel demum reflexis; bracteis interioribus linearibus, tantum 5–7 mm. longis. Flores ligulati 10–14, ligula linearioblanceolati, flavi, 10–13 striis percursi, apice integri, 2.5–3 cm. longi, styliferi; ovario biaristato, aristis sursum hispidulis. Achaenia lineari-oblonga, subnigra, compresso-tetragona, indistincte 16-striata, margine acuto non alata sed valde arrecte tuberculato-ciliata, faciebus non setosa nisi supra, apice dense et arrecte setosa, corpore circ. 5 mm. longa et circ. 1 mm. lata et paleas non aequantia (forsan achaenia omnino matura non vidi?), biaristata; aristis tenuibus, arrecte et breviter hispidulis, 2–2.5 mm. longis.

W. G. Doggett *sine numero*, at altitude of 8000 feet, Mt. Ruwenzori, East Equatorial Africa, comm. in 1901 (type in Herb. Kew).

Bidens rupestris, sp. nov.—Herba erecta, verisimiliter perennis, breviter tomentosa, caule simplici vel parce ramoso, angulato,

internodiis quam foliis multo brevioribus, probabiliter 5–9 cm. alto. Folia petiolata petiolis 0.6–2.5 cm. longis, petiolo adjecto 6–10 cm. longa, pinnata, foliolis lateralibus (circ. 3 jugis) ovatis vel lanceolatis grosse dentatis vel incisis, imis 2–4 cm. longis, dentibus calloso-apiculatis. Capitula radiata, pansa ad anthesin circ. 5–6 cm. lata et 1.2–1.4 cm. alta, breviter tenuiterque pedunculata. Involucri dense hispido-tomentosi bracteae exteriores ± 8, lineares vel lanceolatae, circ. 8 mm. longae, demum reflexae, interioribus aequantes, apice callosae. Flores ligulati 10–16, elliptico-lanceolati, apice obscure denticulati, 2.5–3 cm. longi. Achaenia matura non observata; submatura linearia, brunneo-nigra, valde compressa, faciebus glabra vel supra hispida, margine apiceque sursum setosa, bi (rariter tri)-aristata, aristis infra plerumque spinulis erecte munitis, supra retrorsum 1–3-hamosis vel rariter nudis 2–3.5 mm. longis.—Coreopsidi pinnatipartitae O. Hoffm. habitu valde, similis, sed fructibus maxime diversa.

Dr. C. Uhlig 750, on moss-covered, stony, newly formed volcanic cliffs, "im filzigdichten mannshohen Busch," at altitude of 2400 m., northeast side of Meru Mountain, German East Africa, November 27, 1901 (type in Herb. Berl.).

The type had been determined by Dr. OTTO HOFFMANN as *Coreopsis pinnatipartita* O. Hoffm., but a study of the two type sheets of *C. pinnatipartita* (Herb. Berl.) and other identical specimens (A. Whyte, Nyassaland; Ad. Stolz 1306, etc. in Herb. Berl.) shows that species to have oblong, winged, glabrous, exaristate achenes; not linear, unwinged, upwardly hispid, aristate achenes as in our species.

Bidens Whytei, sp. nov.—Herba erecta, forsan annua (partem inferiorem non vidi), glabra, verisimiliter 4–8 dm. alta, ramis angulatis. Folia subsessilia vel petiolata petiolo lato ciliato 3–12 mm. longoque, petiolo adjecto 1.5–4.5 cm. longa, superiora ac media (infima non vidi) ternati- vel pinnatisecta, segmentis linearioblongis vel lineari-oblanceolatis, integris, apice indurato-apiculatis, subcarnosis, margine plus minusve revolutis spinuloso-ciliatisque, basi angustatis subangustatisve et decurrentibus. Capitula longe ac tenuiter pedunculata pedunculis 5–21 cm. longis et nudis vel 1–3 foliolis foliolata, radiata, ad anthesin 2.5–3 cm. lata et 9–11 mm. alta. Involucrum inferne hispidum; bracteis exterioribus 7–9, brevibus, late spathulatis et supra saepe orbiculato-dilatatis,

apice obtuso apiculatis, sparsim ciliatis, tantum 2–3 mm. longis; interioribus oblongo-lanceolatis, 5–6 mm. longis. Flores ligulati circ. 8, aurei, ligula elliptico-oblanceolati, apice irregulariter dentati, 1–1.5 cm. longi. Achaenia (unum maturum ac multa immatura vidi) linearia, compressa vel plana, exalata, nigra, faciebus 16-sulcata, supra ad margines atque angulos arrecte strigosa, corpore ± 7 mm. longa, biaristata; aristis tenuibus, retrorsum hamosis vel saepe sub medium 1–3 hamis suberectis abnormaliter munitis, circ. 2.5 mm. longis.

Alex. Whyte *sine numero*, near Nairobi (? "Rairabi" on label), British East Africa, received at Kew in August 1903 (type in Herb. Kew).[1]

Bidens Schlechteri, sp. nov.—Herba glabra, gracilis, 5–8 dm. alta, caule parce ramoso, subtetragono, perspicue sulcato. Folia petiolata petiolo 0.7–1.5 cm. longo, petiolo adjecto 5-10 cm. longa, parce membranacea, pinnata vel bipinnata; segmentis linearibus 1.5–4 mm. latis, terminali usque ad 5 cm. longo, margine glabris vel breviter et inconspicue ciliatis, saepe revolutis, apice acuto induratis. Capitula pauca, tenuiter pedunculata pedunculo usque ad 16 cm. longo, radiata, pansa ad anthesin 3–4.5 cm. lata et 7–9 mm. alta. Involucri bracteae exteriores 5–8, lineares, basi hispidae, apice indurato obtusae vel acutae, 4–7 mm. longae; interiores lanceolatae, paulo longiores. Flores ligulati 6–8, flavi, ligula elliptico–ovata, 13–20-striata, apice integra vel obscure denticulata, 1.5–2 cm. longa. Achaenia nigro-brunnea, valde compressa, late oblongo-linearia et utrinque angustata vel moderatim linearia, faciebus leviter sed marginibus apiceque dense erecto-hispida, non vere alata, corpore 5–7 mm. longa et 1.4–2 mm. lata, biaristata aristis circ. 1.5 mm. longis, plerumque nudis, rariter ad apicem una spinula vel erecta vel retrorsa munitis.

R. Schlechter 4745, at altitude of 5000 feet, on slopes of Hortbosch Mountain, Transvaal, March 30, 1894 (type in Herb Berl., two sheets).

Bidens Hoffmannii, sp. nov.—Planta gracilis, glabra, circ. 3–5 dm. alta, caule inferne lignescente, angulato vel obscure tetragono, ramoso. Folia tenuiter petiolata petiolo 4–9 mm. longo, petiolo adjecto 2–5 cm. longa, pinnata, foliolis linearibus 1–3 mm. latis, lateralibus (1 vel 2 jugis) usque ad 2 cm. longis, terminali

[1] For biographic note on WHYTE, see Jour. Bot. 47:155. 1909.

paulo longiore, omnibus integris vel remote pinnato-dentatis, subcarnosis, non nisi petioli basi ciliatis, acute calloso-apiculatis. Capitula pauca, tenuiter pedunculata pedunculis 3–9 cm. longis, radiata, pansa ad anthesin 2.5–3 cm. lata et 6–9 mm. alta. Involucrum moderatim hispidum, bracteis exterioribus circ. 6, linearibus, apice acutis callosisque, 4–6 mm. longis; interioribus lanceolatis, paulo longioribus. Flores ligulati circ. 8, flavi, ligula elliptico-oblongi, apice integri vel acute 2- vel 3-denticulati, 1–1.5 cm. longi. Achaenia subnigra vel cinerea, supra brunnea, compressa, exalata, faciebus 8-sulcata, sursum hispida, saepe tuberculata, linearia, paleas facile superantia, exteriora 6–7 mm. longa et fere 2 mm. lata, interiora 7–9 mm. longa et 1–1.5 mm. lata, omnia biaristata aristis retrorsum hamosis, 1.5–1.75 mm. longis.

G. Volkens 365, at altitude of 1500 m., Kwa Kinabo, Kilimanjaro, German East Africa, June 1893 (type in Herb. Boiss.). Pl. XII.

Recently, through the courtesy of Dr. R. CHODAT, Director of the Institute of Botany of the University, Geneva, I was permitted to study the large collections of *Bidens* in the Boissier herbarium. This and the first following species were found to be new. The plant had been determined at Berlin as a form of *Bidens linearilobа* Oliv. That species, however, is a very different plant, having the exterior involucral bracts 9–13, linear-lanceolate or even narrowly linear, strongly attenuate above, finally more or less reflexed, 6–11 mm. long; also anomalous inner paleae, which are widely elliptic or obovate or truncate, the apex conspicuously and to a great length linear-elongated, surpassing the spreading-awned achenes. The habit is that of *Bidens Kirkii* (Oliv. and Hiern) Sherff, but the fewer heads of *B. Hoffmannii*, the shorter, more often lobed and less parallel sided leaf divisions, and the retrorsely barbed awns of the longer achenes distinguish it very easily from *B. Kirkii*. The species is named in honor of Dr. OTTO HOFFMANN, who not only studied this and other material collected by VOLKENS, but during his lifetime[2] contributed in a most important way to our knowledge of the African species of *Bidens*.

Bidens acutiloba, sp. nov.—Herba erecta, ramosa, verisimiliter annua, caule sulcato, pilis sparsissime consperso, ±6 dm. alto. Folia petiolata petiolis tenuibus 3–15 mm. longis, petiolo adjecto ±6 cm. longa, pinnata, foliolis membranaceis, non dense pilosis, saepe minute atro-punctatis, ovatis vel ovato-lanceolatis, dentatis vel lobatis, dentibus vel lobis acutis, foliolis lateralibus (2-jugis) ±2.5 cm. longis. Capitula corymbosa, radiata, pansa ad anthesin

[2] For an account of his life and work see ASCHERSON, P., Verh. Bot. Ver. Brandenburg **51**:153. 1909; MUSCHLER, R., *ibid.* 155.

2–3.5 cm. lata et 6–10 mm. alta, pedunculis tenuibus, pubescentibus, usque ad 3.5 cm. longis. Involucri bracteae exteriores circ. 8, lineares, dorso hispidae, apice subacutae, 4–7 mm. longae, interioribus lanceolatis parce vel non longiore. Flores ligulati circ. 6, in sicco parce flavi, ligula elliptici vel late oblanceolati, apice minute denticulati, 1.3–1.7 cm. longi. Achaenia atra, linearia, compressa, exalata, superne moderatim latiora, sursum setosa, corpore 5–6 mm. longa et circ. 1 mm. lata, biaristata aristis retrorsum hamosis, 1.5–2.5 mm. longis.

G. Volkens 384, at altitude of 1500 m., Kwa Kinabo, Mt. Kilimanjaro, German East Africa, June 1893 (type in Herb. Berl.).

Bidens kamerunensis, sp. nov.—Herba pubescens, verisimiliter perennis, simplex vel forsan interdum ramosa, 1–1.5 m. alta. Folia petiolata, petiolis latis 0.5–1.5 cm. longis, petiolo adjecto 4–10 cm. longa, 3- (rarius 5-) partita, foliolis lanceolatis, irregulariter crenato-dentatis, dentibus abrupte subcalloso-apiculatis, foliolis lateralibus usque ad 2.5 cm. longis, terminali usque ad 7 cm. longo. Capitula pauca, pedunculata pedunculis subrobustis usque ad 8 cm. longis, radiata, pansa ad anthesin 6–7 cm. lata et 1.2–1.8 cm. alta. Involucri bracteae dense sed breviter hispidae, exteriores 13–16, lineari-lanceolatae, supra sensim attenuatae, inconspicue apiculatae, 1.2–1.5 cm. longae, ad medium plus minusve reflexae; interioribus lanceolatis vel linearibus, interdum brevioribus. Flores ligulati 14–16, lutei, ligula late elliptico-lineares, apice minute denticulati, 2.5–3.5 cm. longi. Paleae anguste lineares, achaenia superantes. Achaenia atra, valde compressa, exalata, sursum hispida vel inferne glabrata, circ. 11–13 mm. longa et 2 mm. lata, saepe biaristata aristis tenuibus, nudis, usque ad 1.5 mm. longis.

C. Ledermann 5461, at altitude of 1200–1400 m., Mao Barkedje, Mt. Gendero, Kamerun, October 6, 1909 (type in Herb. Berl.).

Bidens palustris, sp. nov.—Herba gracilis, erecta, verisimiliter perennis, caulibus plus minusve quadrangularibus, hinc illinc minute hispidis, circ. 5–6 dm. altis. Folia petiolata petiolis 5–12 mm. longis, petiolo adjecto 1.5–6 cm. longa, pinnata vel bipinnata, segmentis linearibus, faciebus glabris, margine sparsim ciliatis, apice acutis, 0.5–1.2 mm. latis et plerumque 1–2 cm. longis. Capitula tenuiter pedunculata pedunculis 5–10 cm. longis, radiata,

pansa ad anthesin circ. 2.5 cm. lata et 6–8 mm. alta. Involucri bracteae exteriores circ. 6, lineares, ciliatae, non aliter nisi sparsim basi pubescentes, versus apicem non conspicue dilatatae, apice acutae, 3–4 mm. longae; interiores lanceolatae, circ. 6–7 mm. longae. Flores ligulati circ. 6, lutei vel fusco-lutei, ligula anguste elliptici, apice acriter dentati, circ. 1–1.2 cm. longi. Achaenia immatura circumambitu triangulato-linearia, compressa, deorsum ex apice angustata, atra, glabra vel ad apicem sparsim erecto-hispida, corpore ad huc circ. 3 mm. longa et 0.8 mm. lata, biaristata aristis 1.5–2 mm. longis, retrorsum hamosis. Planta *Bidenti Kirkii* habitu valde similis, sed differt ligulis, involucri bracteis exterioribus, achaeniis, patria etc.

T. Kassner 2500, in swamps, Kundelungu, Congo Free State, March 13, 1908 (type in Herb. Berl.).

In 1908, T. KASSNER spent at least several weeks among and around the Kundelungu Mountains, southwest of Lake Moero, in southeastern Congo Free State. Among his plants received at the Botanical Museum of Berlin were no less than four new species of *Bidens* and *Coreopsis*.[3] Besides these, he collected (Kassner 2710) the violet-rayed *Bidens urceolata* DeWild., described previously (Ann. Mus. Congo, Bot. IV. **1**:167. 1903) from a specimen (no 464) obtained in 1900 by VERDICK at Lukafu, a locality in this same region; also (Kassner 2850*a*) the red-rayed *Bidens rubra* DeWild., described more recently (Fedde Repert. **13**:203. 1914) from specimens (J. Bequaert 389, Homblé 563) obtained at Welgelegen.

DE WILDEMAN (Ann. Mus. Congo IV. **2**:169. 1913) has referred this plant to *Bidens Kirkii* (Oliv. and Hiern) Sherff (*Coreopsis Kirkii* Oliv. and Hiern). A study of various specimens of *Bidens Kirkii* in European herbaria, however, shows several pronounced distinctions. *Bidens Kirkii* is found several hundred miles farther east, in the coastal region from Mozambique northward to Mt. Kenia; its rays are light yellow, not dark or brownish orange-yellow; its exterior involucral bracts are mostly obtuse at the apex and below the apex noticeably dilated; its immature achenes are not widest at the apex as in *B. palustris*, nor are they practically glabrous, and their aristae are upwardly hispid, with numerous weak hairs, not downwardly barbed with a few spinulose hooks; finally *B. Kirkii* does not appear from collectors' data to be a swamp-inhabiting species as *B. palustris* was observed to be.

Bidens spathulata, sp. nov.—Herba procumbens vel adscendens, plus minusve glabra, caule subsimplici nonnullis nodiis radicante, 3.5–5 dm. longo. Folia non manifeste petiolata, 4–8 cm. longa et

[3] *Bidens palustris*, *B. leptolepis*, *Coreopsis scabrifolia*, and *C. leptoglossa*.

1.5–2.5 cm. lata, valde membranacea, spathulato-oblanceolata, apice subobtusa, inferne sensim angustata, integra vel remotissime minimeque denticulata, ciliata, in sicco plus minusve stramineo-viridia. Capitula perpauca (1–3), pedunculata pedunculis pubescentibus usque ad 6 cm. longis, radiata, pansa ad anthesin circ. 3.5–4.5 cm. lata et 7–9 mm. alta. Involucri bracteae exteriores circ. 6, membranaceae, oblongae vel oblanceolatae, ciliatae, apice orbiculatae, ad anthesin circ. 8–10 mm. longae et 2–3 mm. latae; interiores oblongo-ovatae, apice subacutae, ad anthesin circ. 3.5–4.5 mm. longae. Flores ligulati circ. 6, lutei, ligula elliptico-oblanceolati, apice dentati, 1.5–1.8 cm. longi, 4.5–6.5 mm. lati. Achaenia matura non observata; ovariis juvenilibus valde compressis, oblanceolatis, glabris, 2–2.5 mm. longis et 0.8–1 mm. latis, non manifeste alatis, non aristatis.

Dr. Edgar A. Mearns (Roosevelt Expedition) 1291, at altitude of about 3630 m. in the "giant heath" zone, western slopes of Mt. Kenia, along the trail from West Kenia Forest Station to the summit, British East Africa, September 21–27, 1909 (type in Herb. U.S. Nat. Mus.). Pl. XIII.

A species strikingly different from all other species of *Coreopsis* and *Bidens* and easily distinguished by its thin spatulate leaves. Its habit of rooting at many of the nodes, evidently in response to aqueous conditions of the habitat, is almost as distinctive, being common to very few species of these two genera. As mature or even submature achenes are completely lacking on the type, reference of the species to *Bidens* rather than *Coreopsis* is made on the basis of general habit characters.

Bidens Buchtienii, sp. nov.—Herba annua, erecta, 3–7 dm. alta, caule subtetragono, maximum partem glabro. Folia petiolata petiolis 1–4 cm. longis, petiolo adjecto 3–11 cm. longa, pinnatim 5-partita, foliolis ovatis vel ovato-lanceolatis, dentatis vel terminali saepe subtripartito, dentibus acutis indurato-apiculatis; faciebus inconspicue adpresso-hispidis. Capitula pauca vel numerosa, erecto-pedunculata pedunculis demum plerumque 5–9 cm. longis, discoidea vel interdum radiata radiis 4 vel 5 minimis rudimentariis atro-flavis, ad anthesin circ. 7–8 mm. lata et 5–8 mm. alta, in fructu demum circ. 1–1.8 cm. lata (supra apud achaenia) et 2–2.4 cm. alta. Involucri bracteae hispidae, exteriores 6–9, lineares, apice subacutae vel interdum acerrime apiculatae, demum 5–10 mm. longae et 0.7–1.7 mm. latae, quam interiores lanceolatae vel lineari-

lanceolatae plerumque paulo breviores. Achaenia tenuiter linearia, tetragona, exalata, supra erecto-hispida, maximam partem atra sed versus apicem straminea, biaristata aristis suberectis retrorsum hamosis 2.2–3.3 mm. longis, exteriora corpore 7–12 mm. longa et circ. 1 mm. lata, interiora superne longissime attenuata, circ. 0.8 mm. lata et corpore usque ad 1.9 cm. longa.

Dr. Otto Buchtien 4304, at altitude of 3800 m., La Paz, Bolivia, April 8, 1919 (type and cotype in Herb. Field Mus.); *idem* 708, in fields at altitude of 3750 m., La Paz, February 27, 1907 (Herb. Field Mus., two sheets); *idem, sine num.*, at altitude of 3670 m., La Paz, April 1913 (Herb. Field Mus.); *idem* 814, at altitude of 3750 m., La Paz, March 6, 1919 (Herb. Field Mus.); *idem* 4303 *a* and *b*, at altitude of 3800 m., La Paz, March 23, 1919 (Herb. Field Mus.)

About seven years ago Dr. Otto Buchtien, the eminent South American collector, courteously undertook to secure for me as wide a range as possible of specimens of *Bidens* from South America. His collections since then, as well as his earlier collections, all mainly from Bolivia and Chile, have recently been received and deposited in the Herbarium of Field Museum of Natural History. Not only do some of them throw great light upon heretofore difficult points as to the variations of certain species, but two of them appear to be entirely new to science. The first of these, *B. Buchtienii*, is a robust, much branched annual with leaves somewhat simulating those of *B. scandicina* H.B.K., of *B. odorata* Cav., and of *B. alausensis* H.B.K. The numerous fruiting heads with their elongate achenes are distinctly suggestive of *Cosmos*. The second species is described as follows:

Bidens pseudocosmos, sp. nov.—Herba annua, glabra, ramosa, saltem 4 dm. et verisimiliter usque ad 10 dm. alta, caule ramisque tetragonis. Folia tenuiter petiolata petiolis 0.5–3 cm. longis, petiolo adjecto 2–8 cm. longa, bipinnata, saepe ad petiolorum basim et rarius aliter hinc illinc paucis pilis vestita, foliolis primariis 3–7, ultimis segmentis linearibus, acriter calloso-apiculatis. Capitula ramos terminantia, pedunculis 3–9 cm. longis, discoidea vel obscure subradiata radiis 4–5 minutis albido-luteis, ad anthesin minima, 4–5 mm. alta et circ. 6 mm. lata; demum 2.5–3 cm. alta et 1–2 cm. lata. Involucrum basi hispidum, bracteis exterioribus 6–8, anguste linearibus, ciliatis, apice acute apiculatis, 4–6 (rarius 8) mm. longis, quam interioribus lanceolatis manifeste brevioribus. Paleae maturae 1–1.3 cm. longae, quam achaenia matura multo breviores. Achaenia atra, tenuissime linearia, tetragona, exalata,

supra tenuiter sed sensim elongata et sursum setis stramineis hispida, infra glabra, corpore 1–2.4 cm. longa et 0.4–0.7 mm. lata, biaristata aristis tenuibus erectis divaricatisve stramineis retrorsum hamosis 2–3.5 mm. longis.

Dr. Otto Buchtien 815, at altitude of 3300 m., below Obraje, southeast of La Paz, Bolivia, May 10, 1919 (type, 1st and 2nd sheets, in Herb. Field Mus.).

A species with somewhat the aspect of a *Cosmos*. The flowering heads are those of *Bidens*, however, and the achenes are not abruptly narrowed above into a neck or beak as in typical *Cosmos*. The general habit bears a superficial resemblance to that of certain branched forms of the North American *Bidens tenuisecta* Gray. Apparently the closest ally, however, is *B. Buchtienii*. In fact, the fruiting characters of *B. Buchtienii* are so similar that at first the remarkable habit and foliage differences in *B. pseudocosmos* were thought attributable to the fact that the specimen came from a larger plant than the observed specimens of *B. Buchtienii*. A second sheet of *B. pseudocosmos* was found later, however, bearing a small, entire, fruiting plant and even here both the habit and the foliage distinctions were found to hold true.

Bidens phalangiphylla, sp. nov.—Herba gracilis, forsan perennis, aegre erecta, glabra, usque ad 1 m. alta, caule tetragono, ramoso. Folia longe tenuiterque petiolata petiolis 1.5–4.5 cm. longis, petiolo adjecto 9–15 cm. longe, bipinnatisecta, segmentis primariis paucis remotisque, lateralibus tenuiter petiolulatis, omnibus 3–7-partitis lobis submembranaceis, crebro punctatis, linearibus, acriter apiculatis, non ciliatis, 0.6–3 mm. latis, terminalibus elongatis usque ad 5 cm. longis. Capitula pauca, ramos terminantia, pedunculata pedunculis tenuibus circ. 8–10 cm. longis, radiata, pansa ad anthesin circ. 3 cm. lata et 7–8 mm. alta. Involucri bracteae exteriores circ. 8, lineares, acute calloso-apiculatae, basi subhispidae, 5–7 mm. longae, patentes, interiores lanceolatae 6–8 mm. longae. Flores ligulati circ. 8, lutei, ligula anguste elliptici, apice parce vel raro profunde dentati, 1–1.3 cm. longi. Achaenia nigra, linearia, non elongata, plano-convexa, faciebus glabris circ. 8-sulcata, marginibus superne erecto-ciliata, apice erecto-setosa, corpore 5.5–7 mm. longa et 1.1–1.3 mm. lata, biaristata aristis retrorsum hamosis fere 2 mm. longis.

C. Holst 2967, at altitude of 30 m., Doda, Usambara, German East Africa, June 28, 1893 (type in Herb. Berl.).

A species close to *B. amoena*, but having only about 8 ray florets, the about 8 exterior involucral bracts only 5–7 mm. long and scarcely equalling the interior,

and the achenial aristae downwardly barbed. In *B. amoena* the ray florets number 10–14, the 9–12 exterior bracts are 8–11 mm. long, easily exceeding the interior, and the achenial aristae are erectly hispid. *B. phalangiphylla* is close also to *B. Bequaertii* De Wild., but has the leaves much larger and more openly dissected,[4] narrower exterior involucral bracts, and much smaller fruiting heads with smaller achenes.

Bidens anthemoides (DC.) Sherff, Bot. Gaz. **56**:493. 1913; *ibid.* **59**:306. 1915; *Coreopsis anthemoides* DC., Prodr. **5**:573. 1836; *Bidens sarmentosa* Greenman, Proc. Amer. Acad. **41**:264. 1905; *B. alpina* Brandegee, Zoe **5**:239. 1906–1908; *B. coreopsidioides* Schz. Bip. ex Sherff, Bot. Gaz. **56**:493. 1913; *B. Schaffneri* Schz. Bip. ex Sherff, *loc. cit.*, non *B. Schaffneri* (Gray) Sherff; *B. Purpusorum* Bitter and Petersen ex Bitter, Fedde Rept. **17**:335. 1921.

The varying treatments accorded this species have been discussed in earlier papers (Sherff, *loc. cit.*, 1913 and 1915). Recently Bitter has published the new name *Bidens Purpusorum* Bitt. and Petersen for a plant the ripe achenes of which were collected in 1908 by J. A. Purpus, in fields in the vicinity of Esperanza, State of Puebla, Mexico, and sent to the Bremen Botanical Garden under the name of *Bidens grandiflora* Balb. In the Herbarium of the University of California are two specimens collected by Purpus and bearing essentially similar field data to those given by Bitter. The first one was collected at Esperanza in August 1907, not "1908," and matches Bitter's description well. The second was collected at Esperanza in 1909, and distributed under the name of "*Bidens pilosa* Linn.? Form"; it is Purpus no. 3634. This second plant differs in being more branched and in having merely 3–5-parted leaves, not 2–3-pinnatifid leaves.[5] Both forms have most of the achenial aristae retrorsely barbed, but in this and other respects are connected, however, with typical *Bidens anthemoides* by a large number of herbarium specimens examined by myself. Bitter mentions fruitless searches in the Copenhagen and Berlin (Dahlem) Herbaria among the specimens of Bidens to find one like the Purpus form. This failure to find one is not surprising, since most of the herbarium material of this species has heretofore been filed under *Coreopsis*. Indeed, the Berlin Herbarium has one specimen which, though somewhat immature, is very close to the Purpus plant studied by Bitter: Bourgeau 836, desert near Mexico, tableland, September 8, 1865 (also in Herb. Boissier, Herb. Gray, etc.).

[4] The spreading segments offer a fanciful resemblance to the legs of *Phalangium*, whence the trivial name.

[5] In a number of species of *Bidens* a strong degree of polymorphism is known to occur among the leaves; for example, *B. heterophylla* Ort., *B. triplinervia* H.B.K., *B. andicola* H.B.K., *B. chrysanthemifolia* (H.B.K.) Sherff, and *B. squarrosa* H.B.K. This difference in the leaves, therefore, does not appear to be of much importance in this case.

The type material for *B. anthemoides*, *B. sarmentosa*, and *B. alpina* was in each case the more common small alpine form, 0.5–2 dm. tall. Taller forms are sometimes found, however, a plant collected by HENRY E. SEATON, no. 272, at altitude of 9000 feet on Mt. Orizaba, Mexico, August 8, 1891 (Herb. Univ. Chicago), being over 4 dm. tall. The plants cultivated in Bremen from the achenes sent by PURPUS grew about 5 dm. tall. An exceptional specimen by SCHIEDE from near Tepeyahualco, Mexico (Herb. Berl.), measures over 5.7 dm. in height. In 1917, Professor H. M. HALL of the University of California sent to me from the botanical garden of that university several specimens of this species for determination. He wrote: "It has been in our botanical garden for a long time without name. . . . . It is rather attractive and is being used to some extent as a bedding plant." The largest specimen measured about 4 dm. high, and many of the immature achenes had aristae retrorsely barbed.[6] The occasional presence here of retrorsely barbed aristae in a species usually lacking them demonstrates once again the impracticability of using the presence or absence of such aristae as a distinguishing character for *Coreopsis* and *Bidens* (cf. SHERFF, *loc. cit.*, 1915).

Bidens connata Muhl. var. **fallax** (Warnst.), comb. nov.; *B. tripartita* L. var.? *fallax* Warnst., Verhandl. Bot. Ver. Prov. Brandenburg **31**:157. 1880; *B. decipiens* Warnst., Oesterr. Bot. Zeitschr. **45**:392. 1895; *B. connata* Warnst. *loc. cit.* 475, non exacte Muhl.; *B. connata* var. *fultior* Fern. and St. J., Rhodora **17**:24. 1915.

The European specimens of *B. connata* are so different in general aspect from the more common forms in America that they have been perplexing to botanists. Their primary leaves are less slenderly and distinctly petiolate than in *B. connata* proper, and also fewer-toothed, the teeth being usually larger and more irregular. The outer involucral bracts are distinctly foliaceous, the well developed ones being mostly 3–6 cm. long. Indeed, for some time it seemed to certain European botanists that the European plants represented a new form, and WARNSTORFF did in fact name them *B. tripartita* L. var.? *fallax*, afterwards changing the name to *B. decipiens*. Later, he referred the name *B. decipiens* to *B. connata* Muhl.

In 1915 FERNALD and ST. JOHN (*loc. cit.*) described a variety *fultior* from Rhode Island and Massachusetts. Their specimens (in Herb. Gray) are so closely identical with certain of the European specimens (for example, Heiland 876, Lychen: Schluesoh, Prov. Brandenburg; C. Warnstorf, shore of Lake Neuruppin, Prov. Brandenburg, September 1895; both in Herb. Univ. Vienna) that they can scarcely be distinguished apart. In fact, the only difference which

[6] Dr. HALL has recently examined for me three sheets of material from the same original source (in Herb. Univ. California), and reports that one sheet has exaristate achenes. Thus here, as already seen in many other cases, plants of the same collection are found to vary decidedly in the matter of achenial aristae.

I can find is that several leaves on FERNALD and ST. JOHN's material are tripartite, a variation that is of rather slight importance, since tripartite leaves often occur sporadically in the eastern United States upon the ordinary plants of *B. connata* (cf. SHERFF, BOT. GAZ. **64**:33. 1917). According to the Vienna Code, we must therefore employ the varietal name *fallax* first proposed by WARNSTORFF.[7]

BIDENS PILOSA L. Sp. Pl. 832. 1753; *B. ciliata* Hoffmgg. ex Fisch. and Mey., Ind. Sem. Hort. Petrop. **6**:46. 1839; *B. Montaubani* Phil., Anal. Mus. Nac. Chile, Bot. 1891: 49. 1891.

An original specimen of *Bidens ciliata* Hoffmgg. is in the collection of the younger REICHENBACH at the Museum of Vienna. It was collected by Dr. HOFFMANNSEGG himself. It is a nearly glabrous plant, with discoid heads, and is typical *B. pilosa* L. In the original description it was differentiated from *B. pilosa* because of its having polymorphous leaves, a character of no value here, since *B. pilosa* L., *B. subalternans* DC., and most other species of *Bidens* display a noticeable, sometimes very high degree of polymorphism.

A sheet bearing two type specimens of *Bidens Montaubani* is in Berlin (Herb. Berl.). These were received at Berlin from PHILIPPI himself. They display, in the narrowness of their leaflets, a superficial resemblance to certain specimens of the South American *B. subalternans* DC., but differ in having leaves pinnate, not bipinnate, and the achenes 2-aristate, not 2–4-aristate. A study of their foliage, achenes, and involucres shows that they are to be regarded as mere foliage forms of *B. pilosa* L.[8]

BIDENS TRIPLINERVIA H.B.K. Nov. Gen. et Sp. **4**:231 (182). 1820; *B. hirtella* H.B.K. *loc. cit.* 232 (182); *B. procumbens* H.B.K. *loc. cit.*; *B. crithmifolia* H.B.K. *loc. cit.* 234 (183); *B. delphinifolia* H.B.K. *loc. cit.*; *B. humilis* H.B.K. *loc. cit.* (184); *B. affinis* Klotzsch and Otto, Linnaea **15**:2. 1841; *B. artemisiaefolia* Poepp. and Endl. Nov. Gen. et. Sp. **3**:49. 1845; *B. consolidaefolia* Turcz., Bull. Soc. Nat. Mosc. **24**:185. 1851 (also Ann. Bot. Syst. **5**:225. 1858); *B. humilis* var. *macrantha* Weddell, Chlor. And. **1**:69. 1855; *B. humilis* var. *major* Schz. Bip., Linnaea **34**:528. 1865–1866 (ex num. 52 plantarum Mandonis; nomen subnudum); *B. decomposita* Wall. var. *hirsutior* C. B. Clarke, Compos. Ind. 141. 1876; *B. humilis* var. *tenuifolia* Schz. Bip. ex Grisebach Symb. Fl. Arg.

[7] It might be argued that we should reject all such names advanced with an interrogation, as was *fallax*, but custom appears to decree their acceptance.

[8] Cf. CARLOS REICHE, Fl. Chile **4**:102. 1905: "Difiere de *B. pilosa* por las hojuelas mucho mas angostas i pequeñas; pero las muestras del Museo tal vez son insuficientes para establecer una especie nueva."

198. 1879 (ex syn. *B humilis* var. *macrantha* et num. 52 et pro parte 46 plantarum Mandonis); *B. pilosa* L. var. *discoidea* Schz. Bip. em. 6. *decomposita* f. *hirsutior* O. Ktze. Rev. Gen. 1:322. 1891.

Bidens affinis Kl. and O. was described from specimens raised from seed sent by E. Otto. A number of good specimens are to be found in the older herbaria (Herb. Bernhardi in Herb. Mo. Bot. Gard., Herb. Mus. Vienna, Herb. Par. etc.). Specimens obtained in August 1841 at the Strassburg Botanical Garden and elsewhere from seed sent out from Berlin (where Klotzsch was Keeper of the Royal Herbarium, *fide* Pritzel Thes. 165. 1872) are in the private herbarium of Schultz Bipontinus (in Herb. Par.). These were determined by him as *B. triplinervia* H.B.K. O. E. Schulz (Urban Symb. Antill. 7:143. 1911) likewise equates *B. affinis* with *B. triplinervia*. I myself, in 1914, compared the *B. affinis* specimens with the type and Bonpland duplicate of *B. triplinervia* (Herb. Par.) and found them exactly identical.[9]

In an earlier paper (Bot. Gaz. 61:501. 1916) I have shown that *B. crithmifolia* at times has a simple-leaved state. In 1914, on comparing this simple-leaved spontaneous state—Triana 1374[10] and 1375[11] each in part, also Justin Goudot, ditches, Bogota (Fontibon), February 20, 1844[12]—with the mature *B. affinis* and immature *B. triplinervia* material, I was impressed with the similarity. I searched, but in vain, for any authentic material of *B. affinis* manifesting the divided leaves so much more common than undivided ones on *B. crithmifolia*. Recently, however, through the kindness of Dr. A. Zahlbruckner I have been permitted to study the large set of *Bidens* specimens in the Natural History Museum of Vienna, and have found three more sheets of the original *B. affinis* material. These sheets display numerous gradations from the typical undivided, lanceolate leaves of *B. affinis* to the bipinnate leaves characteristic of *B. crithmifolia*. The plants are a little larger, but this doubtless was due to cultivation. The floral and achenial characters are identical. The species *B. crithmifolia* H.B.K. is seen, therefore, to be synonymous with *B. affinis* Kl. and O. and, with it, to be referable to *B. triplinervia* H.B.K.[13]

B. humilis H.B.K., the type of which came from Equador, is seen from a study of many specimens to equal *B. delphinifolia* and *B. crithmifolia*, and hence likewise must be referred to *B. triplinervia* (cf. Weddell, Chlor. And. 1:69.

[9] The Berlin Herbarium contains two sheets of fine material collected near Angangueo, Mexico, October 1830, by Schiede, also several garden specimens cultivated in 1833 at Berlin, doubtless from seed of the Schiede specimens.

[10] In Herb. Par. and Herb. Brit. Mus.

[11] In Herb. Brit. Mus.

[12] In Herb. Par.

[13] Asa Gray (Proc. Amer. Acad. 22:429–430. 1887) suspected *B. triplinervia* to be a form of *B. heterophylla* Ort. The two species do actually resemble each other in their simple-leaved states, but the shorter achenes of *B. heterophylla* are very distinctive.

1855: "Le *B. crithmifolia* H.B.K. et le *B. delphinifolia* H.B.K. sont très voisins de cette espèce [*B. humilis*], dont ils ne sont même peut-etre que des formes à feuilles moins divisées, et à lanières plus allongées"; also JAMESON, Synops. Pl. Aequator. **2**:117. 1865: "a *B. crithmifolia* et *B. delphinifolia* vix distincta [*B. humilis*] videtur.").

Several original specimens of *B. artemisiaefolia* Poepp. and Endl. are still extant (Herb. Mus. Vienna, Herb. Berl.). They are the form of *B. triplinervia* with tomentose pubescence and 2–3-pinnatifid leaves. They match rather closely the original material of *B. decomposita* var. *hirsutior* C. B. Clarke, and of *B. consolidaefolia* Turcz.

BIDENS HETEROPHYLLA Ortega Hort. Matr. 99. *pl. 12.* 1798; *B. luxurians* Willd. Enum. Hort. Berol. 847. 1809; *B. arguta* var. *luxurians* DC. Prodr. **5**:596. 1836; *B. Warszewicziana* Regel cum vars. α, β, γ, Flora **32**:183–184. 1849.

The extensive synonymy and bibliography of *B. heterophylla* are mostly omitted here, the purpose being to deal mainly with the names *B. luxurians* and *B. Warszewicziana*. The Berlin Herbarium contains three specimens under the name *B. luxurians* Willd. One, from the Kunth Herbarium, was collected at the Berlin Botanical Garden, apparently in 1806,[14] or about three years before the date of publication of *B. luxurians*. A second one was collected in the same garden, but the label bears no date. A third one has a label saying merely "ex horto?" All three specimens are clearly referable to *B. heterophylla* Ort. As at least the first one of these is undoubtedly authentic, since WILLDENOW'S name was founded on the plant growing in the Berlin Botanical Garden, we may unhesitatingly equate *B. luxurians* Willd. (*nec alior*) with *B. heterophylla* Ort.

Bidens Warszewicziana was founded by REGEL upon plants which bloomed in the Botanical Garden at Zurich in the years 1848 and 1849, from seed collected by WARSZEWICZ in the region of Guatemala. Three specimens in the Boissier Herbarium, from the Geneva Botanical Garden, October 1855, are *B. heterophylla* Ort. A specimen (Herb. Univ. Vienna) from the Botanical Garden of Vienna in 1869, obtained from seed sent from the Botanical Garden at Prague in 1868, is likewise *B. heterophylla*. Similarly, a specimen from the Leipsic Botanical Garden in 1857 and several others, mainly from the Berlin Botanical Garden (all in Herb. Berl.), are without exception *B. heterophylla*. REGEL'S description of the species and its varieties shows that he relied largely upon the capricious variations of the foliage for his distinctions, and apparently was unaware that these various forms had already been noted long before under the name *B. heterophylla*, and such synonyms as *B. arguta* H.B.K. and *B. longifolia* DC.

[14] The date is given "1806–12," whence I assume that it was December 1806; or was 1806–1812 intended?

BIDENS ALAUSENSIS H.B.K. Nov. Gen. et Sp. 4:235 (184). 1820; *B. chilensis* var. *apiifolia* DC. Prodr. 5:604. 1836.

In an earlier paper I have already listed *Bidens valparadisiaca* Colla and *B. chilensis* DC. as synonyms of *B. alausensis*. I have since been able to examine a cotype of DE CANDOLLE'S variety *apiifolia* of his *B. chilensis*. It is Poeppig's 207, collected in June in rocky and gravelly places along the coast near Valparaiso, Chile (Herb. Berl.). It is merely an unimportant foliage form of *B. alausensis*, and surely not entitled to separate varietal rank.

Bidens crataegifolia (O. Hoffm.), comb. nov.—*Coreopsis crataegifolia* O. Hoffm., Engler Bot. Jahrb. 30:431. 1901.

For an extended treatment of the bases of distinction between *Coreopsis* and *Bidens* the reader is referred to a former article (SHERFF, BOT. GAZ. 59: 305–309. 1915). In addition to the species transferred from *Coreopsis* to *Bidens* at that time and subsequently, this and the following four species are found to merit a transfer. In each case, I have had the opportunity of carefully studying the type specimens (all in Herb. Berl.).

Bidens Fischeri (O. Hoffm.), comb. nov.—*Coreopsis Fischeri* O. Hoffm., *loc. cit.*

Bidens Stuhlmannii (O. Hoffm.), comb. nov.—*Coreopsis Stuhlmannii* O. Hoffm., *loc. cit.* 415.

Bidens Buchneri (Klatt), comb. nov.—*Coreopsis Buchneri* Klatt, Leopoldina 25:107. 1889 (*non* Ann. Naturh. Hofmus. Wien 7:103. 1892).

Bidens ochracea (O. Hoffm.), comb. nov.—*Coreopsis ochracea* O. Hoffm., Engler Bot. Jahrb. 30:431. 1901.

Bidens paupercula, sp. nov.—Herba annua, erecta, gracillima, glabra, caule subtetragono simplici vel apice parce ramoso, 2–6 dm. alto. Folia petiolata petiolis circ. 8–12 mm. longis, petiolo adjecto 2–4 cm. longa, pinnata, foliolis 3 vel 5, anguste linearibus vel etiam filiformibus, acutis, minute denticuloso-ciliatis, 0.2–0.8 mm. latis. Capitula tenuiter pedunculata pedunculis 8–12 cm. longis, plerumque solitaria, ad anthesin minima, pansa circ. 7 mm. lata et 6 mm. alta, subradiata. Involucri bracteae subaequales; exteriores 4–6, reflexae, lineares, glabrae vel saepius setoso-ciliatae, apice acutae, primum circ. 2 mm. demum. circ. 5–6 mm. longi. Flores ligulati rudimentarii, lutei, lineares, circ. 4–5 mm. longi. Achaenia (circ. 8–15 in singulis capitulis) recta, anguste linearia, compressa, exalata, supra medium sensim usque vel fere ad apicem

angustata, atra, adpresse erecto-setosa, utrinque manifeste 8-sulcata, corpore 1.6–2.2 cm. longa et 0.5–1.2 mm. lata, apice saepe dilatata et semper biaristata aristis tenuibus retrorsum hamosis 1.2–2.5 mm. longis.

Ad. Stolz 1442, in forest at altitude of 900 m., Kyimbila, Nyassaland, July 22, 1912 (two type sheets in Herb. Berl.). Pl. XII.

Unlike most other African species of *Bidens*, this possesses close affinities with *B. bipinnata* L. and *B. parviflora* Willd.

Bidens graciloides, sp. nov.—Herba glabra, erecta, fruticosa, ramosa vel infra simplex, ±5–6 dm. alta. Folia tenuiter petiolata petiolis 1–2.5 cm. longis, petiolo adjecto 3–5 cm. longis, pinnata, foliolis 3 vel 5, ovatis vel ovato-lanceolatis, crenato-serratis, apice obtusis vel acutis, 4–1.2 cm. latis. Capitula laxe subcorymbosa, tenuiter pedunculata pedunculis usque ad 6 cm. longis, radiata, pansa ad anthesin 1.1–1.5 cm. lata et 5–7 mm. alta. Involucri bracteae exteriores 4–6, lineares vel oblongo-lineares, apice obtusae vel acutae, nunc hispidae nunc fere glabrae, 2–3 mm. longae; interiores lanceolatae, dimidio longiores. Flores ligulati circ. 5, flavi, ligula elliptici, apice dentati, 5–7 mm. longi. Achaenia plus minusve arcuata, plana, non vere alata, linearia, marginibus et saepe costis medianis sparsim setosa, exaristata, 6–7 mm. longa et 0.6–1 mm. lata.

C. N. Forbes 2435 O, Waialae Iki, ridge, righthand side, Oahu, Hawaiian Islands, March 2, 1917 (type in Herb. B.P. Bishop Mus.); *idem* 2474 O, ridge between Niu and Wailupe, Oahu, Hawaiian Islands, April 11, 1917 (Herb. B.P. Bishop Mus.).

Bidens diversa, sp. nov.—Herba annua, erecta, gracilis, glabra, ramosa, 3–6 dm. alta, caule tetragono. Folia petiolata petiolis 0.5–2 cm. longis, petiolo adjecto 3–6 cm. longa, bipinnata, segmentis paucis, linearibus, acriter calloso-apiculatis, membranaceis, margine saepe revolutis, 0.3–1.2 mm. latis. Capitula ramos terminantia, tenuiter pedunculata pedunculis usque ad 15 cm. longis, radiata, pansa ad anthesin 8–13 mm. lata et 6–9 mm. alta. Involucri glabri vel subglabri bracteae valde inaequales, exteriores 5–8, lineares, obtuse vel acriter calloso-apiculatae, 1–2 mm. longae, interiores lanceolatae, 4–5 mm. longae. Flores ligulati circ. 6–8, lutei, ligula elliptico-oblongi, apice profunde incisi, 3–7 mm. longi.

Achaenia submatura compressa, atro-brunnea, lineari-oblonga, faciebus glabra, exalata, marginibus et apice erecto-setosa, corpore circ. 5 mm. longa et usque ad 0.7 mm. lata, biaristata, aristis retrorsum hamosis circ. 1 mm. longis.

Antunes 315, in forest at altitude of 1760 m., Mounyino, Portuguese West Africa, March 1901 (two type sheets in Herb. Berl.).

The species may be noted especially for the small flowering heads, the inequality of the exterior and interior involucral bracts, and the sharply and deeply bilobed ligules. It is close to *Bidens ciliata* DeWild., but is easily distinguished by the characters of the rays and of the involucre.

BIDENS INVOLUCRATA (Nutt.) Britt., Bull. Torr. Bot. Club **20**:281. 1893; *Coreopsis involucrata* Nutt., Jour. Phil. Acad. **7**:74. 1834: *Diodonta involucrata* Nutt., Trans. Amer. Phil. Soc. N.S. **7**:361. 1841; *Bidens polylepis* Blake, Proc. Biol. Soc. Wash. **35**:78. 1922.

Recently BLAKE (*loc. cit.*) has published the name *Bidens polylepis* for this species, on the assumption that the name *B. involucrata* had already been occupied by *B. involucrata* Phil. (Anal. Mus. Nac. Chile, Bot. 1891: 49. 1891). An examination of PHILIPPI's original material and description, however, shows that his (PHILIPPI's) species was not a *Bidens* at all, and thus, if the Vienna Code be followed, BRITTON's transfer of NUTTALL's species to *Bidens* under the name *Bidens involucrata* must stand as valid.[15] We may note that OTTO KUNTZE renamed PHILIPPI's species *Heterospermum pinnatum* var. *involucratum* (Rev. Gen. Pl. **3**[II]:158. 1898). FRIES (Rob. E., Alp. Fl. N. Argent. 84. 1905) gives a treatment similar to that of KUNTZE. REICHE (Anal. Univ. Chile **112**:150. 1903; Fl. Chile **4**:99. 1905) likewise placed PHILIPPI's plant with *Heterospermum*, but renamed it *Heterospermum involucratum*.

BIDENS SANDVICENSIS Less., Linnaea **6**:508. 1831; *B. mutica* Nutt., Trans. Amer. Phil. Soc. Ser. II. **7**:368. 1834; *Campylotheca mutica* Walpers Rept. **2**:618. 1843; *Campylotheca sandvicensis* Hillebr. Fl. Haw. Isls. 214. 1888; *Coreopsis sandvicensis* Benth. et Hook. ex Drake del Castillo Ill. Fl. Ins. Mar. Pacif. 210. 1890.

For many years writers have confused various other Hawaiian species of *Bidens* with *B. sandvicensis* Less. Space permits only the more important of such errors to be noticed here. In 1856, MIQUEL (Fl. Ned. **2**:78) equated *B. sandvicensis* with *B. peduncularis* Gaud. In the Berlin Herbarium are

[15] An original specimen given by PHILIPPI to the Berlin Herbarium in 1888 is a small plant with flowering, but not fruiting heads, and matches rather closely in its available characters certain of the forms collected by Dr. OTTO BUCHTIEN under his nos. 243 and 705, at La Paz, Bolivia (Herb. Field Mus.).

certain Sandwich Island (Hawaiian) specimens sent by GAUDICHAUD as *B. peduncularis*, and these specimens are close to *B. sandvicensis*, but the original description of *B. peduncularis* was published by GAUDICHAUD from material collected at Rawak of the far distant Molucca Islands, and which in no way could be mistaken for *B. sandvicensis*.[16] In 1861, GRAY (Proc. Amer. Acad. 5:127–128) gave an extended discussion of *B. sandvicensis*, referring to it *B. mutica* Nutt., *B. gracilis* Nutt., *B. pulchella* (Less.) Schz. Bip. (*Adenolepis pulchella* Less.), etc. The type of *B. mutica* (Herb. Brit. Mus.) matches LESSING'S type of *B. sandvicensis* (Herb. Berl.) very well. *B. gracilis*, however, is an entirely different species (cf. SHERFF, BOT. GAZ. 70:105. 1920). *B. pulchella* is represented by a single small type specimen in Berlin, and is so different in technical characters that by no stretch of the imagination could it be equated with *B. sandvicensis*. Gray (*loc. cit.*) suspected *B. angustifolia* Nutt. of belonging to *B. sandvicensis*, and DRAKE DEL CASTILLO (*loc. cit.*), evidently with GRAY'S treatment at hand, definitely equated the two species. A study, however, of NUTTALL'S type (Herb. Brit. Mus.) shows a plant with more dissected foliage and more slender-awned achenes. Apparently *B. angustifolia* had best be retained, for the present at least, as a separate species.

The following amplified description has been drawn up mainly from LESSING'S type (Adelbert von Chamisso, Oahu, Hawaiian Islands, in 1816, Herb. Berl.), but in small part from Mann and Brigham 541, Waimea, Kauai, Hawaiian Islands, at altitude of 2000–3000 ft. (Herb. B.P. Bishop Mus.).

BIDENS SANDVICENSIS Less., descript. amplif.—Caulis herbaceus, erectus, quadrangulatus, sulcato-striatus, glaber. Folia tenuiter petiolata petiolis usque ad 4 cm. longis, petiolo adjecto 3–12 cm. longa, ternatim vel pinnatim partita, foliolis ovatis vel saepius ovato-lanceolatis, acuminatis, serratis, glabris, membranaceis, subtus pallidioribus, lateralibus manifeste petiolulatis 1–4 cm. longis et 5–12 mm. latis, terminali longiore. Capitula radiata, pansa ad anthesin 11–14 mm. lata et 4–6 mm. alta, in paniculis corymboideis trichotomis subfastigiatis disposita, ramis rigidis folia sua longe excedentibus. Involucri bracteae exteriores 4–6, lineares, glabratae, obtuse acriterve calloso-apiculatae, 2–5 mm.

[16] The Paris specimen (Herb. Mus. Hist. Nat. Paris) of GAUDICHAUD'S is a mere fragment, but is probably from his type material. It matches his description perfectly. It is true *B. chinensis* (L.) Willd. Singularly, Miquel (*loc. cit.*) equated *B. peduncularis* with *B. chinensis*, an equation erroneously rejected by O. E. SCHULZ (Engl. Bot. Jahrb. 50, Suppl.: 179. 1914). GAUDICHAUD'S Paris specimen is labeled as coming from the Sandwich Islands (voyage of the corvette Uranie), but *B. chinensis* is not known to occur in that part of the world, and the specimen may well have come from Rawak as stated by him when he published the original description.

longae, interiores lanceolatae longiores. Flores ligulati circ. 5, lutei, ligula moderate vel late elliptico-oblongi, apice denticulati, 6–8 mm. longi. Achaenia linearia, compressa, exalata, parce curvata, non perspicue attenuata, atra, faciebus glabra et circ. 4-sulcata, marginibus erecto-setosa, corpore 6–9 mm. longa et 0.5–0.8 mm. lata, apice nunc biaristata aristis retrorsum et tantum apice hamosis circ. 1 mm. longis, nunc subaristata vel etiam exaristata sed setis erectis coronata.

Bidens conjuncta, sp. nov.—Caulis ramique herbacei, perspicue quadrangulati, sulcato-striati, glabri, verisimiliter 5–8 dm. alti. Folia tenuiter petiolata petiolis plerumque 2–4.5 cm. longis, petiolo adjecto 5–14 cm. longa, ternatim vel pinnatim 3–5-partita, foliolis glabris, subtus pallidioribus, acriter serratis dentibus perspicue apiculatis, ovato-lanceolatis, acuminatis, lateralibus non petiolulatis sed sessilibus itaque plerumque conjunctis, quam terminali brevioribus. Capitula radiata, pansa ad anthesin 1.2–2 cm. lata et 6–8 mm. alta, in paniculis corymboideis trichotomis disposita, ramis rigidis folia sua longe excedentibus. Involucri bracteae glabrae, exteriores circ. 5, lineares, acutae obtusaeve, patentes vel reflexae, 2–5 vel saepe etiam usque ad 7 mm. longae, interioribus lanceolatis plerumque longiores. Flores ligulati circ. 5, lutei, ligula elliptico-oblanceolati, apice denticulati, 6–10 mm. longi. Achaenia linearia, compressa, exalata, plus minusve curvata, parce attenuata, nigrescentia, faciebus glabra et circ. 4-sulcata, marginibus glabra vel rarius sparsim erecto-setosa, corpore 10-16.5 mm. longa et circ. 1 mm. lata, apice nunc biaristata aristis versus apicem retrorsum hamosis et circ. 1 mm. longis, nunc subaristata vel etiam exaristata sed saepe setis erectis brevibus paucis coronata.

C. N. Forbes 468 M, Honokahau Drainage Basin, Maui, Hawaiian Islands, September 25 to October 17, 1917 (two type sheets in Herb. B.P. Bishop Mus.).

This species, obtained for me by the late C. N. FORBES (cf. SHERFF, BOT. GAZ. **70**:98. 1920), might easily be confused with *Bidens sandvicensis* Less., the general habit of the two being very similar. *B. conjuncta*, however, differs in its larger leaves with sessile lateral leaflets, its larger and more conspicuous external involucral bracts, its longer ray flowers, and its much larger achenes.

Bidens Sereti (De Wild.), comb. nov.—*Coreopsis Sereti* De Wild., Ann. Mus. Congo V. **2**:212. 1907.

There are two ample specimens of the type (Herb. Bruss.). They were collected by F. Seret, no. 306, at the summit of Mt. Angba, on the right bank of the Uele River (Congo), November 9, 1905. The achenes are wingless and their aristae lack barbs. A specimen from Uganda, however, has been found with many of the aristae retrorsely barbed at the apex (pl. XIV, M.T. Dawe 415, at altitude of 5000 feet, Distr. Ankoa, 1905; Herb. Brit. Mus.). This last specimen I had described in my manuscript as a new species, but have since found it to be identical with the type specimens of *Coreopsis Sereti*. It may be remarked that, had De Wildeman relied upon the more significant presence or absence of achenial wings instead of upon the presence or absence of retrorse aristae barbs (cf. SHERFF, BOT. GAZ. **59**:308. 1915), he would have had no hesitation in placing the species in *Bidens* at the outset. As DE WILDEMAN presented no Latin description, the following, drawn from all three specimens examined, is given:

BIDENS SERETI, descript amplif.—Herba perennis, erecta, ± 13 dm. alta, habitu *Heliantho* simillima; caule subtereti vel aegre quadrangulari, glabrescente vel setulis brevissimis hispidulo, non ramoso nisi ad apicem. Folia sessilia, 6–13 cm. longa et 1.5–3 cm. lata, indivisa, lanceolata vel lanceolato-oblonga, rigidula, serrata dentibus calloso-apiculatis, supra rugulosa et glabrata vel scabrido-hispida, infra pallidiora et scabrido-hispida, margine ciliata, apice acuminata, basi inferiora ac mediana sensim summa abrupte angustata, summa saepe etiam subamplexicaulia. Capitula non numerosa, corymbosa, magna, radiata, pansa ad anthesin 5–8 cm. lata et 1.4–1.8 cm. alta, pedunculata pedunculis tomentoso-hispidis, ± 3.5 cm. longis. Involucrum scabrido-hispidum, bracteis imperfecte 4-serialibus, ad apicem saepius reflexis; exterioribus circ. 12 (6+6), subfoliaceis, late lineari-oblongis vel interdum late oblongo-lanceolatis, supra attenuatis, apice acutis, circ. 1.3–1.7 cm. longis, in capite juniore discum facile superantibus; bracteis interioribus late linearibus vel etiam lanceolatis, superne attenuatis, quam exterioribus manifeste brevioribus. Flores ligulata 10–14, flavi, ligula oblanceolati vel elliptico-oblongi, 12–16 striis percursi, tubi apice pilosi, ad summam integri vel interdum lobulati, 2.5–4.5 cm. longi et 0.6–1.5 cm. lati; ovario sterili oblongo vel subcuneato, plano, marginibus et versus apicem arrecte setoso, 2–3 mm. longo. Achaenia matura lineari-oblonga vel subcuneata, plana, subatra, striata, marginibus et apice arrecte setosa, faciebus non setosa nisi

supra, corpore 5–8 mm. longa et 1.4–2 mm. lata, biaristata; aristis tenuibus, suberectis, infra saepe 1–3 setis acriter arrecteque munita, supra calva vel interdum versus apicem 1 vel 2 setis retrorsum hamosis, 1.5–2.5 mm. longis.

Bidens waimeana, sp. nov.—Herba fruticosa, gracilis, erecta, ramosa, glabra, verisimiliter 4–8 dm. alta. Folia petiolata petiolis 1–4 cm. longis, petiolo adjecto 6–9 cm. longa, pinnatim 3–5-partita, foliolis membranaceis, ovato-lanceolatis, acuminatis, serratis. Capitula cymoso-corymbosa vel irregulariter dispersa, radiata, pansa ad anthesin circ. 1.5 cm. lata et 5–6 mm. alta. Involucri bracteae exteriores circ. 5, patentes, lineares, acutae vel obtusae, glabratae vel leviter glanduloso-hispidae, interiores lanceolatas saepe aequantes. Flores ligulati circ. 5, flavi, ligula elliptico-oblanceolati, apice obsolete denticulati, 6-8 mm. longi. Achaenia atra, linearia, compressa, utrinque moderate attenuata, recta vel leviter curvata, non manifeste striata, faciebus rugulosa sed non pubescentia, marginibus suberecto-setosa, corpore 6–8 mm. longa et 0.5–0.75 lata, apice erecto-setosa, saepe ad apicem vel interdum parce sub apicem brevissime biaristata aristis glabris vel rariter 1–2 hamis retrorsum hamosis.

A. A. Heller 2848, on Kaholuamanoa, above Waimea, Island of Kauai, Hawaiian Islands, October 1–8, 1895 (type in Herb. Mo. Bot. Gard., cotype in Herb. New York Bot. Gard.).

A species related to *Bidens gracilis* Nutt., *B. micrantha* Gaud., and *B. micranthoides* Sherff. It can be told from *B. gracilis* by its straight or slightly curved, not twisted achenes; from *B. micrantha*, by its marginally setose, smaller, more slender, and not conspicuously black achenes; from *B. micranthoides* by its taller, more branching, less herbaceous habit. The type specimen has the primary leaves mainly 5-partite; the single cotype examined has them 3-partite.

Bidens Henryi, sp. nov.—Frutex circ. 1 m. altus, glaber, ramis subteretibus. Folia petiolata petiolis tenuibus 2–3 cm. longis, petiolo adjecto 5–11 cm. longa, in specimine typico indivisa, ovato-lanceolata, acuminata, serrata, membranacea. Capitula pauca, pro patria magna, pedunculata pedunculis nudis linearibracteolatisve et 5–7 cm. longis, pansa ad anthesin circ. 4 cm. lata et 1 cm. alta. Involucri glabrati bracteae exteriores circ. 8, lineares, subobtuse apiculatae, 8–10 mm. longae et 0.6–1.2 mm. latae, quam

interiores late oblongo-lanceolatae primum multo demum paulo longiores. Flores ligulati circ. 8, lutei, ligula late lineari-elliptici, 9–13-striati, ad apicem obtusum obsolete denticulati, 1.5–1.9 cm. longi; florum tubulosorum stylo-ramis subrotundis, apice obtusis et non elongato-caudatis. Achaenia anguste vel late linearia, exalata, atra vel interdum terminis brunneo-straminea, faciebus glabra, marginibus glabra vel interdum supra erecte 1–3-setosa, 6–11 mm. longa et 0.7–1.5 mm. lata, apice calva vel irregulariter 1-vel 2-aristata aristis nudis vel obsoletissime hispidis, usque ad 0.6 mm. longis, una saepe altiore quam altera posita.

CH. HENRY, at altitude of 3500–4000 ft., Atuona Valley Ridge on route to Hanamenu, Hiva-Oa Island, Marquis Islands, December, 1917 (type in Herb. Field Mus.).

Bidens Ahnnei, sp. nov.—Frutex circ. 1 m. altus, glaber, ramis subteretibus. Folia petiolata petiolis subalatis 2–3 cm. longis, petiolo adjecto 6–9 cm. longa, ovata vel oblongo-ovata, apice acuta vel subabrupte et breviter acuminata, basi rotundata sed non vere cordata, acriter serrata (singulo latere cum 16–25 dentibus), sicca valde membranacea. Capitula numerosa, parva, corymbosa, pedicellata pedicellis tenuibus breviter lineari-bracteolatis 0.5–2 cm. longis, radiata, pansa ad anthesin 0.7–1.2 cm. lata et 4–5 mm. alta. Involucri bracteae exteriores 5 vel 6, lineares, subacutae et saepe glandulo nitido apiculatae, tergo plerumque glabrae, marginibus ciliatae, 2–3 mm. longae, quam interiores oblongo-ellipticae plerumque paulo breviores. Flores ligulati circ. 5, lutei, ligula elliptico-oblanceolati, apice denticulati, circ. 4–6 mm. longi. Achaenia minuta, exalata, atra, lineari-clavata, infra angustata, circ. 8-sulcata (toto achaenio), marginibus et interdum supra ad costas moderate erecto-setosa, apice exaristata, 3–4 mm. longa et tantum circ. 0.5 mm. lata.—*Bidenti polycephalae* Schz. Bip. affinis.

CH. HENRY, mountain near Hakaui, Nukahiva Island, Marquis Islands, in 1916 (two type sheets in Herb. Field Mus.). The species is named for M. ST. AHNNE, who, as President of the Chamber of Agriculture of Tahiti in 1916, kindly enlisted the services of M. HENRY of Nukahiva in securing for me the type material (cf. SHERFF, BOT. GAZ. **70**:92. 1920).

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EXPLANATION OF PLATES XII–XIV

PLATE XII

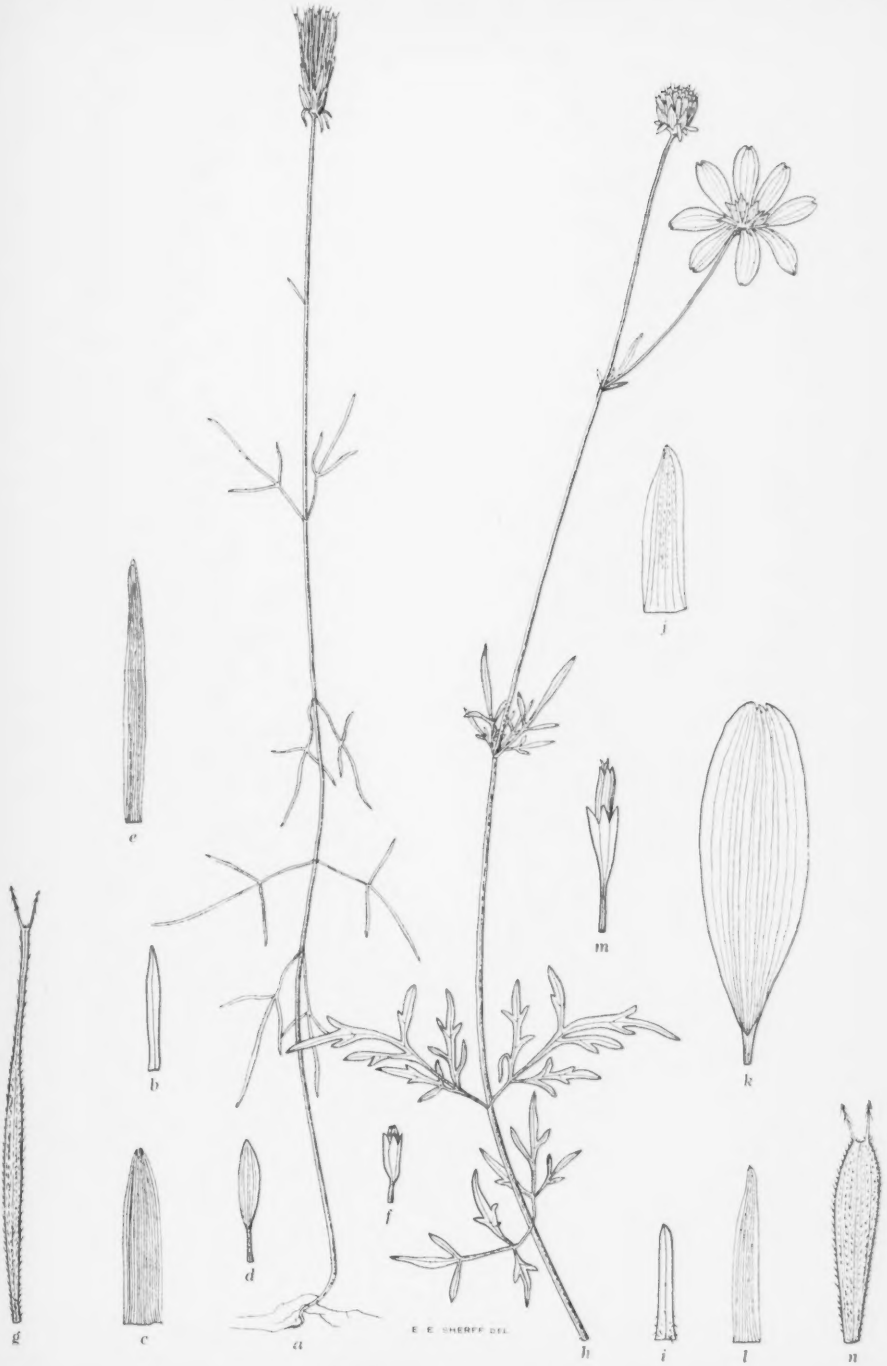
Bidens paupercula: *a*, fruiting specimen, ×0.71; *b*, exterior involucral bract, ×4.25; *c*, interior involucral bract, ×4.25; *d*, ligulate floret, ×4.25; *e*, palea, ×2.8; *f*, disc floret, ×4.25; *g*, achene, ×2.8; all from Ad. Stolz 1442, type, in Herb. Berl; *Bidens Hoffmannii:* *h*, flowering and fruiting specimen, ×0.71; *i*, exterior involucral bract, ×4.25; *j*, interior involucral bract, ×4.25; *k*, ligulate floret, ×4.25; *l*, palea, ×4.25; *m*, disc floret ×4.25; *n*, achene, ×4.25; all from G. Volkens 365, type, in Herb. Boiss.

PLATE XIII

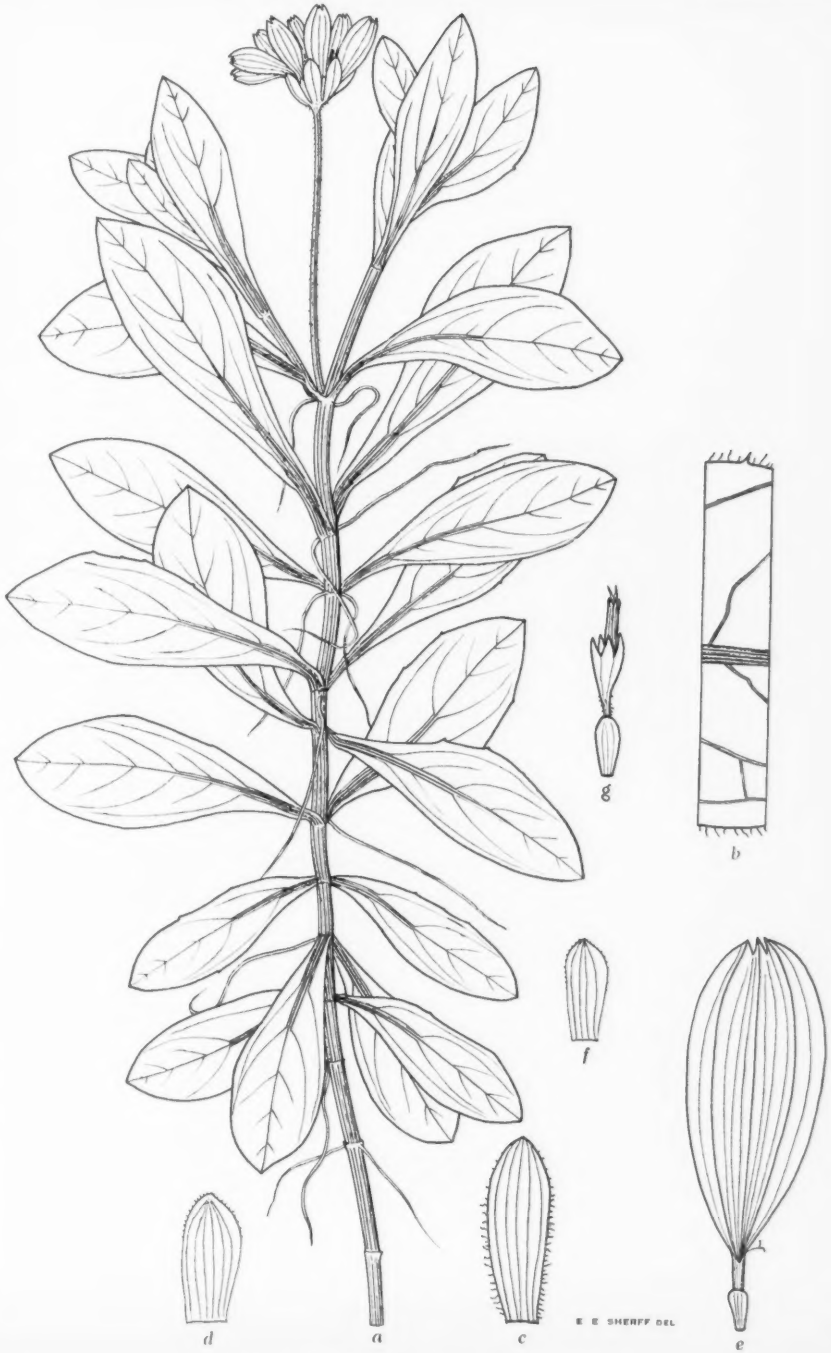
Bidens spathulata: *a*, flowering specimen, ×0.75; *b*, exterior involucral bract, ×3; *c*, interior involucral bract, ×3; *d*, ligulate floret, ×3; *e*, palea, ×3; *f*, disc floret, ×3; all from E. A. Mearns 1291, type, in Herb. U.S. Nat. Mus.

PLATE XIV

Bidens Sereti: *a*, flowering specimen, ×0.61; *b*, portion of leaf, lower surface, ×2.4; *c*, exterior involucral bract, ×1.8; *d*, interior involucral bract, ×1.8; *e*, ligulate floret, ×1.2; *f*, palea, ×1.8; *g*, disc floret, ×1.8; *h*, achene, ×3.6; all from M. T. Dawe 415, in Herb. Brit. Mus.



SHERFF on BIDENS



SHERFF on BIDENS

SHERFF on BIDENS

CHEMICAL CHANGES AT BEGINNING AND ENDING OF REST PERIOD IN APPLE AND PEACH[1]

OUIDA ABBOTT

(WITH SEVEN FIGURES)

Introduction

Periodicity of one kind or another is recognized as a general characteristic of the growth of higher plants. In the temperate zones it suggests the alternation of growing and dormant seasons, and the term "rest period" is often used to refer to the period of dormancy. Strictly speaking, however, it is the dormant and not the rest period that is directly associated with the appearance of seasonal conditions inhibiting growth. In temperate zones dormancy usually begins in the autumn, about the time the leaves fall, and ends in the spring when "growing season" conditions return. On the other hand, the rest period is defined "as that period in which plants are, for the most part, incapable of responding to growing conditions" (HOWARD 6). The plant may be either dormant or in full leaf and yet cannot grow, even though temperature, moisture, and other environmental factors apparently are identical with those under which it grows vigorously at some other time. Dormancy may overlap and extend beyond the rest period, or vice versa, or conceivably they may come at entirely different seasons. Periodicity may be due primarily either to external conditions compelling dormancy, to internal factors associated with the rest period, or to both. The horticulturist, who at times wishes to force and at other times to inhibit the growth of certain plants, is interested no less in the rest period than in dormancy, and sometimes it is essential to distinguish between them. A rest period of longer or shorter duration seems to be general among perennial plants. In trees and shrubs of the temperate zone a cessation of length growth may occur in some or all of their branches in early summer, or even in late spring, although the environment be favor-

[1] Contribution from the Department of Horticulture of the University of Missouri.

able for growth. Some plants rest only a few weeks, others are unable to grow for five or six months. HOWARD (**6**) found the twigs of a single tree began their rest at different times. He believed that the rest period probably began in a given twig shortly after the formation of its terminal bud. Under Missouri conditions, bearing apple trees of average vigor form a large number of terminal buds the latter part of May or early in June. He decided that the deciduous trees and shrubs of the north temperate zone begin their rest period in July, and throughout August and September the rest becomes more intense. JOHANNSEN (**8**) advanced the theory that the rest period is divided into three stages: (1) the early rest, in which the activity of the plant is diminishing; (2) the middle rest, a stage of complete inaction; and (3) the after rest, when development is ready to begin.

HOWARD (**5**) concluded, after experimenting with a number of deciduous plants, that "the secret of the rest period lies in the buds rather than in the cambium, roots, or any of the tissues of trunk or branches." More recent investigations by CURTIS (**2**) and SIMON (**14**) show that the rest period is confined to the one-year old buds that have attained a certain degree of maturity, and to the cambium. It is interesting in this connection that experiments with seeds of many annual plants have shown that when immature they germinate rapidly, but if allowed to ripen they must pass through a resting stage before they can germinate.

FACTORS OF REST PERIOD.—There is a great diversity of opinion among investigators as to the exact nature of the factors that are responsible for the beginning of the rest period. Some consider that the rest period, like dormancy, is brought on and terminated by changing external conditions, in other words, that rest is aitiogenic. Others hold that it is the result of fixed internal and hereditary factors, that it is autogenic. Some investigators have tried to reconcile these different ideas by considering the rest period as due to a "cooperation of an hereditary tendency to rhythm with the after effects of periodic repetition of reactions induced by external conditions" (PFEFFER **13**). Probably, if environment is important in initiating or ending the rest period, it acts by influencing certain fundamental internal processes, and it is the changes in

these internal processes that are the factors more directly associated with the inception and end of the rest period.

WEBER (16) has attempted to correlate the rest period with permeability. He quotes the following from FITTING (3):

> The permeability of the epidermal cells of *Rheo discolor* for various salts varies with the season. In winter the permeability of many leaves is practically nil, but in summer the permeability is high. *Rheo discolor* possesses a well marked rest period from November until spring.

In 1914, KREHAN (10) arrived at the same conclusion as FITTING, working with the same plant but using different methods. Both FITTING and KREHAN concluded that cells of the resting buds of temperate zone trees and shrubs may be assumed to have a decreased permeability for certain substances.

HOWARD'S (6) investigations led him to conclude that the beginning of the rest period is associated with the inhibition of enzyme action, due to the over-accumulation of the products of their activity, and that "the specific effect of all rest breaking agents on resting woody tissue is the stimulation of the enzymes." COVILLE (1), working with the blueberry, advanced the theory that the chilling of resting plants which is followed by growth is associated with the transformation of starch into sugar. He supposes that during rest the starch grains are separated by the living active cell membranes from the enzymes that would transform starch into sugars, and that when the plant is chilled the "vital activity" of the cell membrane is weakened so that the enzymes "leak" through it and change the starch into sugars. This hypothesis associates the breaking of the rest period with both increased permeability and increased enzyme activity. Presumably, according to this conception, decreased permeability and an accompanying inhibition of enzyme activity would be associated with its beginning. Parenthetically, it may be stated that no evidence has ever been presented to show that starch-splitting enzymes "leak in" or "leak out" of the cell. According to the accepted theory of enzyme activity, the same enzyme that hydrolizes will, under suitable conditions, synthesize. The factor that really determines the formation or the disintegration of starch is the local concentration of sugar, which depends on the amount of sugar supplied the regions of storage. Since the

manufacture of sugars proceeds during the summer before as well as after the time when starch storage occurs, storage is evidently more closely associated with carbohydrate utilization than with carbohydrate manufacture. During the active growing season, although the synthesis of sugars is going on, but little starch is formed, since the sugar is used before it can be changed into starch. When growth ceases or is retarded and carbohydrate utilization is correspondingly reduced, then starch is formed. The enzymes that change starch into sugar are present at all times, and merely accelerate a reaction that could in time take place without them. It seems plausible that the enzymes which most directly affect the rest period are not the starch-splitting enzymes but certain others, perhaps oxidases, involved in the utilization of carbohydrates.

THE PROBLEM.—Apparently investigators have given little consideration to the chemical changes taking place in resting tissues at the beginning and end of the rest period, although the explanations offered by HOWARD (**6**) and COVILLE (1) suggest that such changes may be of considerable importance. This investigation deals with the effects of certain treatments on young peach and apple trees in delaying or hastening the inception or the end of the rest period, and includes a study of certain chemical changes within the plant associated therewith.

Material and methods

In an attempt to find chemical differences between resting and non-resting tissues, samples of the tips of one-year old seedling peaches, and the tips and bark of four-year old Grimes apples were collected for analysis. These portions were selected as being most indicative of the differences in chemical composition of resting and non-resting tissues. All of these trees were growing at the Fruit Farm near Turner Station, Missouri. The peach samples were taken from a plot of 1000 one-year old seedlings of fairly uniform height and size. The apple samples were collected from four-year old Grimes trees. These trees were all under cultivation, were in good condition, and the apples had received careful treatment as regards pruning and spraying. Each time a sample was taken from a tree, the entire tree was heavily pruned, and observations were

made as to the effect of pruning on subsequent growth. The samples were collected from June 30 to October 25 at intervals of about ten days. For the sake of uniformity all samples were collected between nine and eleven o'clock in the morning. The fresh weight of the samples was usually between 40 and 50 gm. At intervals of ten days, from July 7 to December 1, samples were collected and H-ion determinations run.

Observations and chemical determinations were made also on a number of standard seedling peach trees, semi-dwarf, and true dwarf peach trees that were grown in pots and then taken into the greenhouse at regular intervals during the winter. From time to time collections were made of shoots and spurs of different apple varieties. These were treated in various ways and then placed in jars of water in a warm room. Suitable records were made of the way in which these twigs responded to growth promoting conditions.

The determinations made include moisture, reducing sugars, total sugars, starch, total carbohydrates, water soluble pentosans, and H-ion concentration. The methods for determining moisture, reducing sugars, total sugars, starch, and total carbohydrates are given in Research Bulletin 40 of the Missouri Agricultural Experiment Station (7).

Water soluble pentosans were estimated according to the following procedure. Three grams of dry, powdered tissue was placed in a 400 cc. beaker, which was filled about half full of water and allowed to stand ten minutes. The material was then boiled for ten minutes and filtered while still hot. The residue was washed four or five times with boiling water. The filtrate and washings were caught in a 700 cc. pyrex flask, 8 cc. of concentrated hydrochloric acid added, and the flask was then connected with a reflux condenser and boiled for three hours. After cooling and neutralizing with sodium hydroxide, the solution was placed in a 400 cc. beaker and yeast ground with a small quantity of water added. The beaker was incubated at 40° C. for twenty-four hours. The yeast destroys all the reducing sugars except pentoses, which are produced as hydrolytic products of the pentosans. After the material was removed from the oven it was filtered and the precipitate washed two or three times. The solution was then boiled

to remove the alcohol and to reduce the volume. The solution was then transferred to a 250 cc. volumetric flask cleared with basic lead acetate, deleaded, and analyzed for reducing sugar in the manner indicated (7). The official Munson-Walker method of analysis was used to determine the reducing power of the sugars. Fifty cc. of each solution was used. The reduced copper was collected on ashless filter paper, burned, and the residue weighed as copper oxide.

GILLASPIE'S (4) colorimetric method was used for all H-ion determinations. The fresh material was reduced to a pulp and the sap extracted with a small amount of distilled water. The H-ion concentration was determined by matching the color of the plant extract with the standards. All determinations were run in duplicate, and each number represents an average of two analyses that checked.

Results

EFFECTS OF PRUNING AND OTHER TREATMENTS ON REST PERIOD.—The condition of the young peach and apple trees when the samples were taken, and observations on the trees at intervals after pruning are given in tables I–III. These tables show that growth in length in

TABLE I

CONDITION OF TREES WHEN SAMPLES WERE TAKEN

Date	Peach	Apple
June 30	Growing	Growing
July 8	Growing	Growing
July 28	Growing	Slow growth
August 4	Growing	Slow growth
August 12	Growing	Slow growth
September 16	No new growth	Terminal buds forming
September 25	Terminal buds formed	Abscission layer formed, leaves falling
October 6	No growth	Adventitious buds and immature terminal buds growing
October 28	No growth	Drop in temperature, no growth

the unpruned trees ceased about the end of September, and that trees pruned September 25 or later made no secondary growth. On the other hand, apple and peach trees pruned June 30 to August 6 made a very luxuriant secondary growth, while trees pruned August 6 to September 15 made only a feeble secondary growth.

TABLE II

OBSERVATIONS ON PRUNED GRIMES TREES FROM JUNE 30 TO OCTOBER 15

DATE OF OBSERVATION	DATE OF PRUNING									
	June 30	July 8	July 28	August 4	August 12	September 6	September 16	September 25	October 6	October 15
June 30	Trees in vigorous growth									
July 8	Growth started well over the tree									
July 28	Growth average 20 cm.	Growth average 8 cm.								
August 4	Growth average 50 cm.	Growth average 25 cm.	Growth average 10 cm.							
August 12	All growing vigorously				Trees growing					
September 6	All growing vigorously					Terminal buds forming; abscission layer formed				
September 16	Trees still growing					Only two or three buds breaking	Terminal buds formed; no new growth			
September 25	Trees still growing						Growth feeble	No new growth		
October 6	Trees still growing							Growth feeble	No new growth except adventitious buds	
October 15	Terminal buds formed; adventitious buds and immature terminal buds breaking									Adventitious and terminal buds breaking

These trees evidently were entering their initial or early rest in late August and early September.

Owing to the high temperature and heavy rainfall from October 6 to October 15, there was a general breaking, in both the unpruned trees and in those pruned from August 6 to September 25, of the immature terminal buds and of latent buds near the crotches. There was no evidence, however, that any resting buds began activity at this time. This corroborates the findings of SIMON and

TABLE III

OBSERVATIONS ON PRUNED PEACHES FROM JUNE 30 TO OCTOBER 15

DATE OF OBSERVATION	DATE OF PRUNING				
	June 30	July 8	July 19	August 12	September 25
June 30	Growing vigorously				
July 8	Growth started well all over tree				
July 19	Growth 40–50 cm.	Growth started			
August 4	Growth 40–50 cm.	Growth 20–24 cm	Growth 6–8 cm.		
August 12	Growth 12–60 cm.				
September 6	Trees still growing			Growth 5–6 cm.; terminal buds forming on unpruned trees	
September 25	Trees still growing			Growth 10–12 cm.	Terminal buds formed on all unpruned trees
October 6	Growth much slower; terminal buds forming			Growth 12–14 cm.	No new growth
October 15	Terminal buds formed				No new growth

others already cited, that buds do not enter a resting stage until a certain degree of development has been attained.

Table IV gives observation on seedling peaches treated August 1, and on standards, dwarfs, and semi-dwarfs potted July 29 and placed in the greenhouse to see the effect on the rest period of continued summer temperatures and abundant moisture. The standards lost their leaves by November 1 and the dwarfs by December 1. Both began growing again about January 1, and a few blossoms were opening on the dwarfs January 25. The semi-dwarfs did not lose their leaves until late in January, and it was a month later before they began to grow again. These trees evidently

TABLE IV

OBSERVATIONS ON PEACHES (SEEDLINGS, STANDARDS, DWARFS AND SEMI-DWARFS) POTTED JULY 29, PLACED IN GREENHOUSE SEPTEMBER 23

DATE	SEEDLINGS TREATED AUGUST 1			PLANTS POTTED JULY 29, PLACED IN GREENHOUSE SEPTEMBER 23			
	Defoliated	Pruned	Dark box	Standards	Dwarfs	Semi-dwarf	Check
September 23.....	New leaves formed	Growth 6–14 cm.	.....	.....	.....	.....	Terminal buds formed
October 1.....	Growing	Growth 10–20 cm.	.....	Leaves falling	Terminal buds formed	Terminal buds formed	No growth
November 1.....	No new growth, leaves green	Terminal buds formed, no further growth	Dead	Leaves off	Leaves green	Leaves green	Leaves fallen
December 1.....	Terminal buds formed, leaves green	Tree bare	.....	Tree bare	Tree bare	Leaves green	Tree bare
January 1.....	Terminal buds formed, leaves green	Tree bare	.....	Growth started	Growth started	Leaves green	Tree bare
January 25.....	Growth started	Tree bare	.....	Growth 5–10 cm.	Blooming	Losing leaves	Growth started
February 1.....	Growth 5–8 cm.	Growth started	.....	Growth 10–20 cm.	Peaches set	Trees bare	Growth 8–10 cm.
February 25.....	Growth 10–12 cm.	Growth 6–8 cm.	.....	Growth 15–25 cm.	Peaches well set	Growth begun	Growth 15–20 cm.

were all in the rest period when taken into the greenhouse. The high temperature and favorable growing conditions greatly prolonged the predormant period of the semi-dwarfs. It greatly shortened the period of dormancy in all cases. Apparently, in the light of results reported later on the effect of cold in breaking the rest period, the greenhouse treatment prolonged the rest period of the semi-dwarfs, while its influence on the standards and dwarfs is uncertain.

Table V is a record of a number of standard peach seedlings, potted September 25 and taken into the greenhouse, one every fifteen days, from October 1 to February 25. These data are

TABLE V

Taken in greenhouse	Minimum temperature for previous fifteen days (degrees F.)	Began growing
October 15	50	March 1
November 1	36	March 1
November 15	17	February 15
December 1	25	February 15
December 15	15	January 3
January 1	7	January 10
January 15	12	January 30
February 1	− 3	February 6
February 15	7	February 20

presented graphically in fig. 1, and show that the breaking of the rest period was hastened by exposure to low temperatures. Thus trees brought into the greenhouse October 15 remained dormant four and one-half months, while trees brought in January 1, after exposure to a temperature of 7° F., began growing within ten days. In this instance the rest period had been shortened forty-eight days by severe freezing.

During January, twigs were cut from trees of a number of early and late blossoming varieties of apples, and placed in jars of water in the greenhouse to see whether possibly a late blossoming variety remains in a resting state later in the spring than an earlier blossoming one. The varieties used were Oldenburg, Ralls, York, Rome, and Jonathan. No appreciable difference was found in the time of opening of the flower buds between the early and the late blossoming varieties. The Oldenburg began to grow five or six days before

the others, but otherwise the differences were slight. The Rome and Jonathan began growing about the same time, yet in Columbia, Missouri, there is an average of six days' difference in their blossoming date out of doors. This, however, may indicate that the rest period of all of the varieties studied had terminated before the samples were taken for study, or it may mean that the wounding incident to cutting them was in itself a rest breaking agent. The experiment suggests, however, that the difference in time of blossoming of early and late blossoming apple varieties, in a section like Missouri, with its severe winters, is not due primarily to differences in the duration of their rest periods.

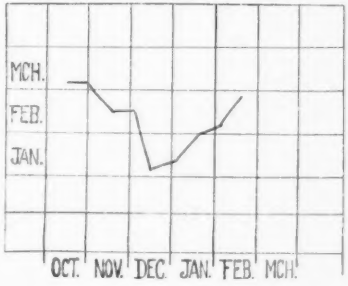


FIG. 1.—Effect of freezing on shortening rest period of young peaches; date plants were placed in greenhouse shown on abscissae, date when growth being shown on ordinates.

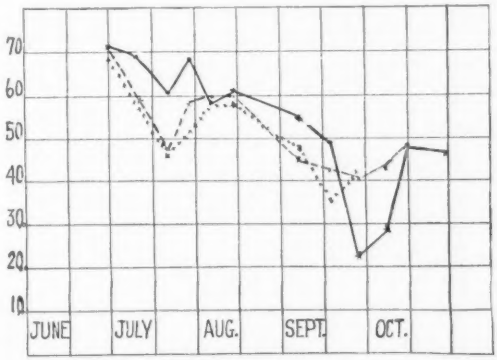


FIG. 2.—Percentages of moisture in young peach tips and apple tips and bark: solid line, apple tips; broken line, apple bark; crosses, peach tips.

CHEMICAL CHANGES AT BEGINNING AND END OF REST PERIOD.—*Carbohydrates.*—The percentages of moisture, reducing sugars, non-reducing sugars, starch, total carbohydrates, and water soluble pentosans for apple tips and bark and for peach tips are given in

table VI, and are shown graphically in figs. 2–6. The curves for dry weight (fig. 2) show minima for both apple bark and tips and peach tips in September. The greatest drop occurs in the apple

TABLE VI

WATER AND CARBOHYDRATE CONTENT (IN TERMS OF DRY WEIGHT) OF APPLE AND PEACH TIPS AND APPLE BARK

Material	June 30	July 8	July 15	July 19	July 28	Aug. 4	Aug. 12	Sept. 6	Sept. 16	Sept. 25	Oct. 6	Oct. 15	Oct. 25
	Water content in percentages of fresh weight												
Apple tips	72	69	65	61	68	58	61	55	49	23	29	47	46
Apple bark	69	59	50	47	58	59	59	45	42	40	43	48	.....
Peach tips	68	.....	.....	56	.....	58	58	47	35	42	42	43	.....
	Reducing sugars (as glucose)												
Apple tips	.....	0	0	0	0	1.34	2.04	2.06	.....	2.45	.....	2.50	2.35
Peach tips	.....	0	0	0	0	1.31	1.34	1.35	.....	1.80	.....	2.03	1.87
	Non-reducing sugars												
Apple tips	.....	.....	.....	0.84	.....	Trace	.....	.....	.....	Trace	.....	Trace	Trace
Apple bark	.....	.....	.....	0.89	.....	Trace	.....	.....	.....	Trace	.....	Trace	Trace
Peach tips	.....	.....	.....	1.71	.....	2.91	.....	.....	.....	2.97	.....	4.91	4.25
	Starch (as glucose)												
Apple tips	.....	.....	.....	0.70	.....	1.89	.....	.....	.....	4.20	.....	4.56	4.26
Apple bark	.....	.....	.....	0.85	.....	1.90	.....	.....	.....	4.37	.....	4.05	4.20
Peach tips	.....	.....	.....	0.83	.....	1.03	.....	.....	.....	3.95	.....	3.80	3.04
	Carbohydrates (as glucose)												
Apple tips	.....	.....	.....	18.45	.....	20.63	.....	.....	25.25	.....	25.88	.....	25.95
Apple bark	.....	.....	.....	18.53	.....	20.26	.....	.....	25.75	.....	20.08	.....	20.65
Peach tips	.....	.....	.....	19.54	.....	20.03	.....	.....	21.25	.....	21.88	.....	21.89
	Water-soluble pentosans (as glucose)												
Apple tips	.....	.....	.....	1.85	.....	1.78	.....	.....	.....	3.25	.....	3.45	3.43
Apple bark	.....	.....	.....	1.89	.....	1.80	.....	.....	.....	3.00	.....	3.27	3.29
Peach tips	.....	.....	.....	1.00	.....	1.85	.....	.....	.....	2.25	.....	4.41	4.39

tips and the least in the peach tips. The minima for the moisture curves occurred in July, with the highest point in the apple tips and the lowest in the peach tips. Figs. 3–6 show an increase in all carbohydrates from July to October in both the apple and the

peach, although between July and October only traces of non-reducing sugars were found in the apple tissues. HOOKER has reported a maximum of non-reducing sugars in bearing apple spurs

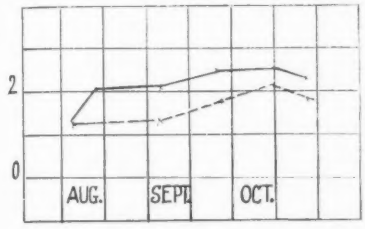


FIG. 3.—Variation of reducing sugars in percentages of dry weight: solid line, apple tips; broken line, peach tips.

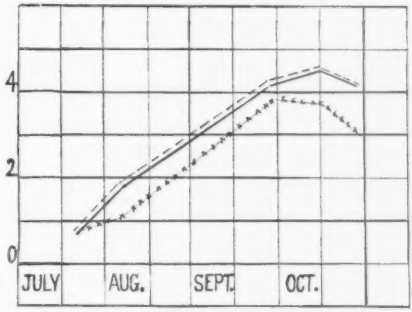


FIG. 4.—Variation in percentages of starch in young peach and apple tips: solid line, apple tips; broken line, apple bark; crosses, peach tips.

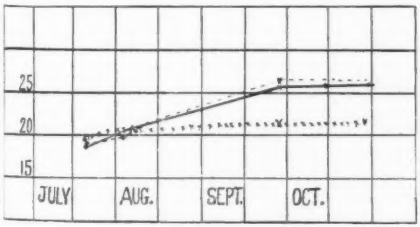


FIG. 5.—Variation of total carbohydrates in percentages of dry weight: solid line, apple tips; broken line, apple bark; crosses, peach tips.

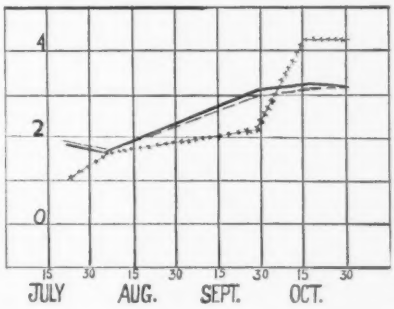


FIG. 6.—Variation of water soluble pentosans in percentages of dry weight: solid line, apple tips; broken line, apple bark; crosses, peach tips.

in November, and in January in non-bearing and barren spurs. In this connection it may be pointed out that the early summer pruning treatments which delayed the beginning of the rest period would result temporarily in a lower carbohydrate content in the tissues near

the pruning cuts. This is significant in connection with JOST'S (9) observations that defoliation or placing trees in a dark box in midsummer delays the beginning of the rest period, for these treatments would lead to a reduction in carbohydrate content. In just what way the accumulation of carbohydrates and the beginning of the rest period are related cannot be stated, but they are associated phenomena.

H-ion concentration.—The H-ion concentration for apple and peach tips is given in table VII, and is shown graphically in fig. 7. The data show that it increased from July to September in both the

TABLE VII

P_H OF APPLE AND PEACH TIPS

Date	Apple tips	Peach tips
July 8	5.40	6.10
July 15	4.50	5.40
July 28	4.80	.....
August 4	4.85	5.80
August 12	4.83	4.85
September 6	4.65	4.85
September 15	4.80	.....
September 25	4.95	4.20
October 6	4.95	.....
October 15	4.65	4.65
November 16	5.89	5.85
December 15	5.85	5.85
January 4	5.85	5.85
January 15	5.85	5.94
February 3	5.90	6.21
March 2	6.00	6.10

peach and apple tips, reaching a maximum in September. It decreased again in October in the apple tips, when a new flush of growth took place from latent and immature buds. During the winter months there is comparatively little change in H-ion concentration, but the initiation of new growth is accompanied or preceded by another decrease. This substantiates MITRA'S (12) work in which he found that "the H-ion concentration is high in summer and low in winter. In general during the growing season the roots, leaves, bark, and tips of the apple seedlings are distinctly acid, while during the dormant period they approach close to neutrality."

A possibly significant relationship between the high H-ion concentration found in this investigation at the inception of the rest period and the inhibition of enzyme activity found by HOWARD (6) to be similarly associated with the beginning of the resting condition is suggested by the work of SHERMAN (15), who found that H-ion concentration had a marked effect on the activity of certain plant enzymes. It is also interesting that MARSH (11) found a parallelism between the changes in sulphur content and those in H-ion concentration to exist in many tissues of the apple during the greater part of the year. A maximum in the sulphur curve occurs in apple spurs in September, when there is a maximum H-ion concentration. His work suggests that at this particular time, and in general when sulphur is high, a considerable percentage of the sulphur is in the form of sulphate. As sulphates dissociate more readily than most other kations found in plant tissues, and since the amount of sulphur present in apple spurs in September is not inconsiderable, being greater than the phosphorus content, it does not seem improbable that the high H-ion concentration at the inception of the rest period may be directly associated with the high sulphur content.

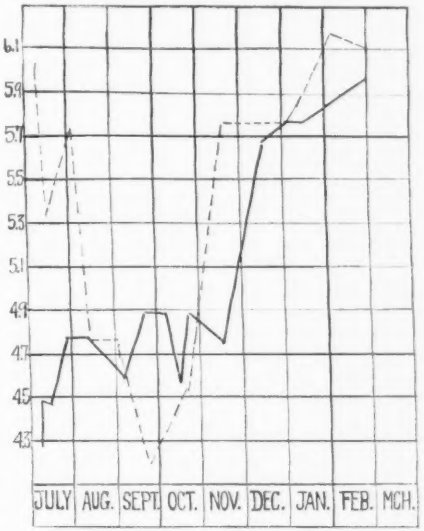


FIG. 7.—Variation in H-ion concentration measured as P_H in young peach and apple tips: solid line, apple tips; broken line, peach tips.

In analyses of fruit buds of the Belle of Georgia and Conover varieties of peach, HOOKER found that nitrogen and phosphorus increase from very low percentages on October 3 to considerable

amounts on January 24 (table VIII), but that phosphorus increases much more rapidly than nitrogen. As all organic phosphorus-containing compounds likewise contain nitrogen, this leads to the inference that much of the increase in phosphorus is due to phosphates. It seems probable, therefore, since an inverse relation

TABLE VIII

CHANGES IN PHOSPHOROUS CONTENT OF PEACH BUDS DURING DORMANT SEASON (UNPUBLISHED DATA BY HOOKER)

Material	Dry weight (percentage)	Phosphorus (percentage dry weight)	Nitrogen (percentage dry weight)
	October 3		
Belle of Georgia			
Distal	49.0	0.093	1.46
Basal	48.0	0.080	1.35
Conover			
Distal	50.0	0.108	1.55
Basal	49.5	0.101	1.39
	December 11		
Belle of Georgia			
Distal	51.3	0.214	.....
Basal	51.0	0.196	.....
Conover			
Distal	49.1	0.241	.....
Basal	49.0	0.216	.....
	January 24		
Belle of Georgia			
Distal	46.4	0.370	1.70
Basal	45.0	0.254	1.63
Conover			
Distal	47.5	0.270	1.68
Basal	59.5	0.270	1.68

between H-ion concentration and phosphate content holds during a great part of the year in certain plant tissues, that in them, as in blood, phosphates may be the buffer substances that regulate H-ion concentration. The high H-ion concentration at the beginning of the rest period resembles a condition of acidosis; at least there is an

interesting parallelism between the conditions of acidosis in plants and in animals, as in both cases the utilization of sugars seems to be inhibited.

Discussion

In the light of the results of this and earlier investigations, it is suggested that fundamentally the rest period problem should be attacked from the same point of view as problems in nutrition. Its inception and its end are associated with changing relations in H-ion concentrations, carbohydrate content, and the relative content of certain inorganic salts, particularly sulphates and phosphates. Within certain limits these relationships can be changed by cultural treatments of many kinds. It might be sometimes directly through sulphate or phosphate supply, sometimes more indirectly through factors influencing carbohydrate manufacture, or sometimes still more indirectly through factors influencing carbohydrate utilization that the rest period may be most readily hastened, delayed, prolonged, or shortened. The limits within which it may be influenced, the exact methods to employ, and their practicability from the point of view of making more profitable the culture of the plant in question, constitute a problem that must be worked out for each different crop or ornamental plant. The results of this investigation and the data here assembled suggest points of departure for the scientific solution of such problems.

To Messrs. H. D. Hooker, Jr., V. R. Gardner, and F. C. Bradford of the department of horticulture of the University of Missouri the writer is greatly indebted for many helpful suggestions and constructive criticism, and wishes to acknowledge gratefully this assistance.

University of Missouri
Columbia, Mo.

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LEAFLETS OF CYCADACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 312

SISTER M. ALICE LAMB

(WITH PLATES XV, XVI)

Introduction

The Cycadaceae are usually determined by the character of the sporophyll; but since the strobili are not always present, keys based upon gross topography and the histology of the leaflets would often be helpful. This study was undertaken to determine whether genera and perhaps species of Cycads might be determined by the leaflets.

The Cycads at the University of Chicago, representing eight genera and thirty species, all growing luxuriantly, were at my disposal. Of most species there were several plants of different ages; and in addition to the living material, there were herbarium specimens of seventeen species, representing six genera from the Royal Botanical Gardens at Kew, three species from the New York Botanical Gardens, and one species from Lincoln Park Conservatory, Chicago. For the anatomical study, sections of mature leaflets were prepared by the paraffin and freehand methods. *Encephalartos caffer*, *E. Altensteinii*, *Zamia integrifolia*, and other species presented difficulty in infiltration, making it necessary to resort to freehand sections, which however were cut sufficiently thin to show details of structure. The sections were stained in safranin and light green, or safranin, gentian violet, and orange G.

Most of the work on Cycadaceae has dealt with the strobili and stem anatomy; comparatively little work has dealt with the anatomy of the leaf. KRAUS (3) described the tissues of the Cycad leaflet in general (the leaf parenchyma, epidermis, hypodermis, chlorophyllose tissue, and mucilage canals), giving a more or less detailed description of twenty-five species in six genera. NESTLER (8), in his work on eight genera, devoted considerable attention to the distribution of stomata on the pinnules and rachis. He dis-

agreed with KRAUS, who noted that stomata occasionally appear on the upper side and at the base of a leaflet. NESTLER did not observe stomata on the upper epidermis of the leaflets of any of the twenty-two species he studied except *Bowenia spectabilis*, a hitherto unmentioned species. He also discusses at considerable length the extent and development of the hypodermis and palisade tissue, and the occurrence of calcium oxalate crystals in several genera. Neither in the work of KRAUS nor NESTLER is there any description of *Microcycas*. LIGNIER (**4, 5, 6**) has two articles on the venation of Cycadaceae which he describes as dichotomous, and one on the venation and transfusion tissue of *Cycas;* and MIQUEL (**7**) has given a brief description of vernation in the family. POOLE (**9**), in a recent investigation, compares the leaves of the modern Cycadaceae with those of their ancient relatives.

Topography

A careful survey of the material in the greenhouses revealed gross characters which readily distinguished six of the nine genera of Cycadaceae. *Cycas* and *Stangeria* are the only forms having a midrib, and these two are easily distinguished, as *Stangeria* has conspicuous side veins, while *Cycas* has none. From the genera having no midrib *Bowenia* is easily separated by its bipinnate character, and *Macrozamia* by the gland borne at the base of each leaflet. Of the five remaining genera, two have a characteristic leaf attachment. In *Dioon* the bases of the leaflets are exceptionally broad, either meeting or overlapping on either side of the rachis. *Microcycas* has the leaflets reflexed on the rachis. The three remaining genera, *Encephalartos*, *Ceratozamia*, and *Zamia* are not so easily disposed of. The attachment of the pinnules on either side of a conspicuous ridge on the abaxial surface of the rachis is a character common to all the available species of *Encephalartos*, but it is shared with *Ceratozamia* and some species of *Zamia*. The gradual decrease in the size of the pinnules toward the base of the rachis, and final reduction to two regular rows of spines reaching to the base of the rachis, is a character of the mature plants of *Encephalartos* only. As the available plants of *E. Lehmanni* are young, this reduction in the size of the pinnules is not so pronounced, nor are the two rows of spines evident. Juvenile plants of *Dioon* show a

similar reduction in the size of the basal leaflets. In *Ceratozamia* and *Zamia* there is a slight reduction in the size of the lower pinnules, but the spines, when they do occur, are scattered irregularly over the base of the rachis, and do not represent reduced leaflets. In adult plants, therefore, its greatly reduced leaflets would separate *Encephalartos* from *Ceratozamia* and *Zamia*. Vernation, when it can be observed, is perhaps the better character on which to separate *Zamia* from *Encephalartos* and *Ceratozamia*. In the two latter genera the vernation of the midrib is erect; in the former it is subcircinate. The pinnules of *Cycas* are circinate. The rachis of the new leaf is erect when it has attained a length of 12 cm. or more, but in fronds 4–8 cm., as in *C. revoluta* and *C. media*, the vernation in the rachis is inflexed (subcircinate). Vernation in the sporophylls of *C. media* was also noted. The tips of the ovulate sporophylls are decidedly circinate; those of the staminate are subcircinate. When vernation cannot be observed, the species of mature *Zamia* and *Ceratozamia* can generally be distinguished by trunk characters. Nearly all species of mature *Zamia* have scarcely any trunk above ground, while *Ceratozamia* establishes a very definite aerial trunk at a very early period. A leaf character which, although not decisive in itself, gives emphasis to a decision already reached might be given here. *Ceratozamia* leaflets are always entire, while in *Zamia* the leaflets are either entire or more or less serrate, at least at the tip.

The following key, based upon these characters and further characters of vernation, may be used for determining genera.

(a) Leaflets with midrib
  (b) Rachis erect in unexpanded leaves; circinate vernation in the leaflets; no side veins.....*Cycas*
  (b) Rachis subcircinate; leaflets straight; side veins.....*Stangeria*
(a) Leaflets without midrib
  (c) Once pinnate
    (d) Rachis subcircinate; pinnules straight in unexpanded leaves; leaflets entire or irregular in outline; trunk generally underground, so that the leaf bases do not form a persistent armor.....*Zamia*
    (d) Rachis and pinnules erect in unexpanded leaves
      (e) Gland present at the base of each leaflet.....*Macrozamia*
      (e) No glands on the leaf
        (f) Very broad attachment of leaflets to the rachis...*Dioon*

(f) Leaflets conspicuously narrowed above the place of attachment; lower leaflets more and more reduced until they are represented, at the base by two rows of spines *Encephalartos*

(f) Leaflets narrowed at the base, but the lower leaflets not reduced to spines. . . . . *Ceratozamia*

(d) Vernation not known; leaflets reflexed on the rachis. . *Microcycas*

(c) Twice pinnate; rachis and pinnae subcircinate. . . . . *Bowenia*

Histology

The anatomy of the leaflets is typically xerophytic. The epidermis is strongly cutinized, and has sunken stomata which are confined to the under surface, except in *Bowenia* and *Macrozamia*. The stiff, leathery character of the leaves is due in a large measure to the thick-walled hypodermal cells, which occur in most species, either as a continuous tissue or in ridges above and beneath the vascular bundles. The mesophyll may be differentiated into a more or less distinct upper and lower palisade, or upper palisade only, and spongy parenchyma; or may show no such differentiation. The cells of the spongy tissue vary from almost isodiametric cells to cells conspicuously elongated, parallel with the leaf surfaces. Both the palisade and the spongy tissues contain chlorophyll. In *Cycas* the midrib is traversed by a single vascular bundle, and in *Stangeria* by several. In the former, beneath the palisade on either side of the midrib, the so-called "transfusion tissue" occurs. In *Stangeria* the side veins are visible in the mesophyll. In the other seven genera the spongy parenchyma is traversed by many bundles, which branch dichotomously but appear parallel, and are inclosed by a bundle sheath of slightly thickened cells, sometimes interspersed with bast fibers, or by a strong sheath of sclerenchymatous cells. Conspicuous mucilage canals occur in the mesophyll or midrib in four of the nine genera. Bast fibers and crystals of calcium oxalate are scattered through the palisade and mesophyll of some of the genera.

Microcycas

The monotypic genus *Microcycas* was used for detailed drawing, because to my knowledge it has never been figured, and is not even mentioned by either Kraus or Nestler. The leaflet is not one of

the most xerophytic, but will serve to show the general xerophytic character of the family. The epidermis is strongly cutinized (figs. 1, 2). Thick-walled hypodermal tissue occurs as an intermittent layer of one, two, or rarely three cells in thickness beneath the upper epidermis, as a constant tissue at the revolute edge of the leaf, and as conspicuous ribs in connection with the vascular bundles. Bast cells form the bundle sheath and join the sclerenchymatous ribs above and below the bundle. The mesophyll is differentiated into (1) a layer of broad and not very elongated palisade-like cells beneath the upper epidermis; and (2) a spongy tissue composed of a region of large, irregular cells, and one of cells transversely elongated, their length being from three to five times their width. No mucilage ducts or crystals were found in this leaf. The sunken stomata are quite numerous in the lower epidermis.

Bowenia

Bowenia, with its broad thin leaflets, showed the least xerophytic characters of any species studied (fig. 3). The epidermis is heavily cutinized, but there is no thick-walled hypodermal tissue. The mesophyll is differentiated into an upper region of two layers of cells compactly arranged but rarely if ever elongated, and a spongy tissue of isodiametric cells or of cells only a trifle elongated. Bast fibers occur singly in the spongy tissue. The vascular bundles are inclosed in bundle sheaths of thin-walled cells. The stomata, which are not deeply sunken, appear on both surfaces of the leaflet, but on the upper surface are fewer and are restricted to the bases of the pinnules.

Stangeria

Stangeria, a genus which may be monotypic, was studied in the well known species *S. paradoxa* (fig. 4). The cutin is thick and the epidermal cells heavy-walled, but there is no differentiated hypodermal tissue except in the midrib and at the revolute edge of the leaf. A double layer of closely arranged and scarcely elongated cells occurs beneath the upper epidermis, but is not found in the midrib, where its place is taken by sclerenchyma. The mesophyll cells are smaller and more compact near the lower epidermis than near the middle of the leaf. The stomata, as one would expect in

the absence of a differentiated hypodermis, are not deeply sunken. They open into large air chambers in the mesophyll. In the midrib sclerenchymatous cells occur as a layer of one, two, or three cells in thickness beneath the upper and lower epidermis; they are scattered in groups through narrow regions beneath and above the upper and lower epidermis, and singly through the rest of the midrib. Six, seven, or eight fibrovascular bundles are found in the midrib, each surrounded by a bundle sheath, which however is not very distinct. The cells of the ground tissue in the midrib are rounded. The small mucilage canals are found only in the midrib. Traces of the side veins, of course, are seen in the blade on either side of the midrib. Crystals of calcium oxalate are present in the mesophyll.

Cycas

The species of *Cycas* studied were *C. revoluta*, *C. circinalis* (fig. 5), and *C. media*. Like *Stangeria*, this genus has a midrib, but, unlike *Stangeria*, it has no side veins and only one bundle in the midrib. The surface is highly cutinized in the species examined, and the thick-walled epidermal cells are conspicuously pitted. In *C. revoluta* they are almost isodiametric on the upper surface and rectangular on the under, the longer axis being perpendicular; in *C. circinalis* they are transversely elongated, except in the midrib region where they are almost isodiametric; in *C. media* they are quite regularly so. The extent of the hypodermis varies in the species. In *C. revoluta* its thickness beneath the upper epidermis is one or two cells, except over the midrib where it is three or four; while on the under surface the hypodermis occurs only in the midrib, where it is one or two cell thick. At the exceedingly revolute margin the hypodermis, here usually three cells deep, is very thick-walled. In *C. circinalis* the hypodermal tissue, two or three cells in thickness in the midrib, is entirely wanting in the tissue on either side of the midrib, and again occurs at the edge of the leaf, where it is one cell in thickness. All the species have an upper palisade, which at the revolute edges of the leaflets is continuous with a lower palisade-like region of less elongated and compactly arranged cells. In *C. revoluta* both the upper palisade and the lower palisade-like layer pass through the midrib, in *C. circinalis* the upper palisade

only, and in *C. media* neither. In all the species the fibrovascular bundle is surrounded by a sheath, which is most conspicuous in *C. media*, where the inner walls of the sheath cells are very heavily lignified. Bast fibers are also found in the midrib and as a rule encircle the bundle sheath. The ground tissue of this region is composed of spherical cells with slightly thickened walls. A peculiar feature of the genus is the occurrence of the so-called "transfusion tissue" in the mesophyll. It consists of elongated, empty, pitted cells which do the work of xylem, and of the cells resembling phloem which perhaps aid in the transfer of food. Large sunken stomata are very abundant on the lower surface of the leaf except in the midrib region. The very thick-walled epidermal cells around the stoma extend downward, forming a domelike chimney. At the base of this are the two buffer cells and guard cells which, when open, allow the air to pass into the chamber beyond. The usual crystals of calcium oxalate are abundant in the midrib of the leaflets of these species.

Ceratozamia

Two species of *Ceratozamia*, *C. longifolia* (fig. 6) and *C. mexicana* were available for sectioning. These two species, if they are in reality distinct species, are very similar; in fact, the only difference observable in the many living specimens examined was that the leaflets of *C. longifolia* are longer than those of *C. mexicana*. Even this is not a constant character, as some of the plants bear leaflets which are not long enough for *C. longifolia* and too long for *C. mexicana;* and leaves of the distinct *C. mexicana* and *C. longifolia* types are found on the same plant. The structure of the leaflets of the two species was also found to be almost identical. The leaf surfaces are heavily cutinized. The thick-walled epidermal cells on the under side are larger than those on the upper side of the leaflet. In the fully matured leaf the hypodermal tissue beneath the upper epidermis forms a layer one cell in thickness, almost continuous in *C. longifolia* and intermittent in *C. mexicana*. It is from two to six cells in thickness at the revolute edge, and two or three cells on the under side, where it forms ridges beneath the bundles. The mesophyll is sharply differentiated into an upper palisade of very regularly arranged cells, and a loose spongy tissue

of cells transversely elongated to about five times their width. The cells of the spongy tissue to some extent show the peculiar arrangement figured for *Zamia furfuracea* (fig. 12). Where the ends of the cells of the spongy tissue come together, bast cells are formed, which, before they become thickened, closely resemble small mucilage canals. Each fibrovascular bundle is encircled by a bundle sheath and an outer circle of bast cells. Sunken stomata occur on the lower surface but are not numerous. Calcium oxalate crystals are present around the vascular bundles and in the mesophyll.

Macrozamia

Macrozamia Moorei was the only species of the genus sectioned. The most striking feature in the leaf was the presence of stomata on both surfaces of the leaflet (figs. 7, 8). The general habit of the plant, the leaves being almost erect until they become mature, perhaps accounts for such a distribution of stomata in this species. Of the twenty-two species of Cycads examined by Nestler, *Bowenia spectabilis* is the only one reported to have stomata on both surfaces of the pinnae. *Macrozamia Moorei*, however, was not among the species worked. As both surfaces of the young leaf are exposed to practically the same conditions, the cuticle, epidermis, hypodermis, and palisade are about equally developed on the upper and lower sides of the leaflet (fig. 7). The vascular bundles, which are encircled by a sheath of sclerenchymatous and bast cells, alternate with the large mucilage ducts. Suberized cells occur scattered among the rounded cells of the spongy tissue.

Dioon

Dioon edule and *D. spinulosum* (fig. 9), as seen in transverse sections, look very much alike. Outside of the heavy cuticle is a coating of bloom which gives the leaf its glaucous character. This coating, however, does not show in the sections as our present method of fixing and staining dissolves it. The epidermal cells on the upper surface are smaller than those on the lower, but in both cases they are much thickened. The hypodermis on the upper side of the leaf in both species is one or two cells in thickness. On the under side of the leaf of *D. edule* it occurs only under the vascular

bundle, where it is two or three cells in thickness. In *D. spinulosum* it is quite steadily one cell in thickness. The differentiation of the mesophyll is equally distinct in both species. The palisade cells are from four to six times as long as broad, while the loose spongy tissue is composed of cells rounded or only slightly elongated. The very deeply sunken stomata are numerous on the lower surface of the pinnae, and open into conspicuous air chambers. Bast cells form a sheath around the bundles, and are found in the spongy tissue in both species, but their presence in the palisade was observed only in *D. edule*. Crystals of calcium oxalate are more abundant in the spongy tissue of *D. edule* than of *D. spinulosum*. In both species the mucilage canals occur above the vascular bundles.

ENCEPHALARTOS

Leaflets of *Encephalartos villosus*, *E. Hildebrantii*, *E. Lehmanni*, *E. Altensteinii* (fig. 10), *E. caffer*, *E. cycadifolius*, and *E. brachyphyllus* were sectioned. In all the species examined a coating of cutin, a thick-walled epidermis, hypodermal tissue to a greater or less extent, an upper palisade one cell deep, crystals of calcium oxalate in the spongy tissue, and numerous stomata in the lower epidermis are constant features. In all these species except *E. brachyphyllus* and *E. Lehmanni* suberized cells encircle the bundle, and in all except *E. Altensteinii* occur nowhere else. *E. Hildebrantii* and *E. villosus* are the only two of the examined species not having mucilage canals alternating with the vascular bundles. *E. Lehmanni* has a lower as well as an upper palisade.

E. Hildebrantii, for a time regarded as a variety of *E. villosus*, but now classed as a distinct species, resembles the latter in leaf structure, as it does in general habit. In fact, the only difference detected in the cross-sections of the leaflets of the two species was a very slight and perhaps inconstant one, that is, the cells of the spongy tissue of *E. Hildebrantii* are a trifle more elongated than those of *E. villosus*. The heavily cutinized epidermal cells are small and their walls are not greatly thickened. The thickness of the hypodermal tissue on the upper side of the leaf is one or two cells, while on the lower surface it is quite constantly one cell. The ratio of length to breadth in the palisade cells is two and one half

to three, and in the spongy cells, two to five. In both of these species crystals are very abundant in the vascular bundle region.

E. caffer and *E. Altensteinii* look very much alike in general habit. The leaflets of both are spiny in the juvenile condition, but *E. caffer* loses its spines sooner, and produces larger cones than does *E. Altensteinii*. Although the xerophytic characters are better developed in *E. Altensteinii* leaflets, yet their structure is very similar, as would be expected from a consideration of the general features of the leaves of the two species. The upper hypodermis, although of about the same depth in both species (two or three cells), is a little thicker walled in *E. Altensteinii*. In this species the lower hypodermis is two or three cells deep between the bundles and four or five underneath them; while in *E. caffer* it is one or two cells deep between the bundles and three cells deep beneath them. The walls of the lower hypodermis are thicker in *E. Altensteinii* than in *E. caffer;* the cells beneath the bundles in the latter have scarcely any lumina. The palisade cells in the latter species are three to five times as long as wide, and in the former are a trifle broader, but not quite as long. The cells of the spongy tissue are from two to five times as long as wide in both species, and show the bridling character to some extent (fig. 12). The deeply sunken stomata are more numerous in *E. Altensteinii* than in *E. caffer,* but the other characters of the leaf, the distribution and structure of bundles and canals, and the occurrence of many crystals and bast cells in the spongy tissue, are the same in both species. The bundles are separated by a distance about equal to the thickness of the leaflet.

E. Lehmanii is the only one of the seven species examined having a lower palisade, the cells of which, however, are only about one-third as elongated as those of the upper palisade. The epidermal cells are smaller than the cells of the hypodermis, which on the upper side of the leaf is two cells, on the lower one cell, and at the revolute edge three or four cells in thickness. Both epidermis and hypodermis are thick-walled. The cells of the spongy tissue are elongated and have the bridling arrangement previously mentioned. Crystals occur near the bundles, which are separated by a distance a little greater than the width of the leaflet. The mucilage canals are small and occupy a position midway between, but a little above, the bundles.

In *E. cycadifolius* the mucilage canals are conspicuously large, their circumference being about as great as that of a bundle including the ring of bast cells encircling it. The cells of the spongy tissue are only about one and one-half to two times as long as wide; the cells of the palisade are broad, and their length is only about three times their breadth. The hypodermis on the upper side of the leaf is one cell, or rarely two cells in thickness; on the lower side it is one cell between the bundles and one or two cells beneath them. Neither epidermal nor hypodermal cells have greatly thickened walls, and on the lower side of the leaf the hypodermis is conspicuous only beneath the bundles.

E. brachyphyllus has a very thick cutin, especially on the upper side of the leaf. The epidermal cells are smaller than the hypodermal. Although the hypodermis has a thickness of one cell (or rarely of two cells) beneath the upper epidermis, two cells at the edge of the leaf; on the lower surface one cell between the bundles, and two or three cells underneath the bundles, the leaf does not appear very xerophytic, as these hypodermal cells, especially those on the lower side of the leaf, are large and not correspondingly thick-walled. The cells of the palisade are broad, their length being only twice their breadth. The spongy tissue is loose and elongated. The mucilage ducts are as large as the bundles, which are inclosed by a sheath of slightly thickened cells.

Zamia

Zamia pumila, *Z. floridana*, *Z. Ottonis* (fig. 11), *Z. integrifolia*, *Z. furfuracea*, *Z. portoricensis*, and two as yet undescribed species were sectioned. Of these eight species all except *Z. integrifolia* and *Z.* II (one of the unnamed species) show the hypodermis continuous with the sclerenchymatous cells surrounding the bundles, as shown in the diagram. *Z. integrifolia* is the only one of the species examined in which sclerenchymatous cells are found scattered through the mesophyll. In all the available species the mesophyll is differentiated into a palisade or a palisade-like layer and a spongy tissue. The usual crystals of calcium oxalate are present in all the species, but no mucilage canals appear. A heavy cuticle, thick-walled epidermal cells, and cells of spongy tissue elongated to from three to five or six times their breadth, are

further features common to all the species studied. The stomata are not so deeply sunken as in species having a heavier epidermis.

In *Z. pumila* the hypodermis appears beneath the upper epidermis only as an occasional cell, except over the bundles, where it extends to the bast cells encircling the bundle. On the under side it occurs only in the little pad of cells which are continuous with the bundle sheath and the bast cells; at the revolute edge of the leaf it is three cells deep. The cells of the upper epidermis are smaller than those of the lower. The palisade-like tissue is generally only one layer of cells, although in some places it is two. The cells are very broad, and those in the lower layer are loosely arranged. *Z. floridana* looks much the same as *Z. pumila* in sections, the chief distinction being a continuous one-celled, or in a few places a two-celled hypodermis beneath the upper epidermis between the bundles.

A cross-section of *Z. integrifolia* might very easily be confused with one of *Ceratozamia mexicana*. The sections of *Z. integrifolia* examined, unlike *Z. mexicana*, showed no hypodermal tissue except at the edge of the leaf, where it is one or two cells deep. The epidermis is thick-walled. As in *Z. mexicana*, the cells of the palisade, although not more than about two times as long as broad, form a distinct region. The cells of spongy tissue in *Z. integrifolia* are not so elongated as those of *C. mexicana*, but the arrangement of cells is the same. Bast cells are formed in the spongy tissue as described under *Ceratozamia mexicana*.

Z. furfuracea has the most xerophytic structure of any of the species examined. The epidermal cells are small, but have very thick walls. The hypodermis beneath the upper epidermis is a continuous layer one or two cells in thickness in the region between the bundles, while it extends down to meet the sheath in the bundle region. The hypodermal tissue is extensively developed beneath the bundles, but does not occur on the under side of the leaf between the bundles. The mesophyll is differentiated into a palisade of two and in some places three layers of elongated cells, and a very loose spongy tissue of much elongated cells. The bast cells around the bundles are numerous. The bridling character of the spongy tissue is shown in fig. 12.

In *Z.* I (the undescribed species of *Zamia* found near Tuxtepec, Mexico) the hypodermis is extensive over and beneath the bundles,

is four or five cells in thickness at the revolute edge of the leaf, and appears irregularly beneath the upper epidermis between the bundles. The bast cells above the bundles are so suberized that the lumina are scarcely visible. The palisade consists of two layers of broad cells whose length is only about one and one half times their breadth. The bridle-shaped cells of the spongy tissue are elongated to as much as eight times their breadth.

Besides the usual hypodermal cells in the region of the fibro-vascular bundles, *Z. Ottonis* has an intermittent layer one cell thick beneath the upper epidermis, and a layer three or four cells thick at the revolute edge of the leaf. The epidermis is very thick-walled. The palisade is not well differentiated; it is made up of two, in places three layers of very broad cells. The spongy cells, with the exception of one or two layers next to the lower epidermis, are elongated to from three to six times their breadth and are bridling to some extent.

Z. portoricensis has the hypodermis only above and below the vascular bundles, and at the revolute edge of the leaf, where it is only one cell thick. The cells of both epidermis and hypodermis have large lumina. The mesophyll is differentiated into a palisade-like tissue two or three cells deep and a large spongy tissue of cells elongated to about six times their breadth.

The other unnamed species of *Zamia*, *Z.* II (which, as previously stated, was found growing in a *Ceratozamia* region), does not show the dipping of the thick-walled hypodermis which is so pronounced in six of the species studied, although a little pad of differentiated hypodermis one or two cells in thickness does occur beneath the bundles. The only other thick-walled hypodermal tissue occurs at the edge of the leaf, where it is about two cells in thickness. The palisade-like cells are scarcely elongated, but since the spongy cells are transversely elongated to four or five times their breadth, the differentiation of mesophyll is very distinct. The thickness of the epidermis is pretty well restricted to the outer and lateral walls.

Ecology of leaflet

In considering the structure of the leaflets, the age of the leaf and the environmental factors must be taken into account. While this study was in progress many of the plants under observation were

forming new crowns, so that leaflets of all ages were available. *Dioon edule*, *D. spinulosum*, and *Encephalartos villosus* are extremely villous in the bud, but as the leaflets unfold this character becomes less conspicuous. The young leaflets of *D. spinulosum* when fully expanded are covered with a heavy bloom, and show no trace of hairiness; those of *D. edule* and *E. villosus* are villous to a slight degree. *Cycas revoluta*, *C. media*, *C. circinalis*, *Zamia pumila*, *Z. floridana*, and *Z.* II are more or less hairy in the bud. The mature leaflets of the Cycadaceae studied, with the exception of *Encephalartos brachyphyllus* and *E. cycadifolius*, are free from hair, at least on the upper surface. The freshly unfolded leaflets of all the species examined are soft, and are easily distorted by contact with any object, such as another plant or a fence.

The leaflets sectioned were all taken from plants which had their growth under the favorable conditions found in a greenhouse. The plants either were raised from seedlings or had been transferred from the field at least ten years before. Their xerophytic structures, therefore, probably were not developed as extensively as they would have been in plants grown under the hard conditions of their natural habitat.

How far environment has influenced the structural development cannot be stated, as no leaflets taken from plants growing in the field were sectioned for comparison. The influence of environment on habit, however, is clearly illustrated by the behavior of *Stangeria paradoxa*. This species has two distinct forms, the one growing in the open, and the other in the shade of larger plants. The leaf of the latter, the bush veldt form, is two times as large as that of the former, the grass veldt form. Specimens of the grass veldt form were sent to the University of Chicago greenhouse, where they gradually assumed the habit of the bush veldt form.

The more important environmental factors of the natural habitat of Cycadaceae are temperature, moisture, and light. The Cycads are all tropical or subtropical. *Zamia floridana* and *Z. pumila* are the only subtropical species of the western hemisphere. In the eastern hemisphere *Macrozamia Moorei* is found at Springsure in the Tropic of Capricorn; *Cycas revoluta* is most luxuriant in the vicinity of Kagoshima, in southern Japan, north of the Tropic

of Cancer; *Cycas circinalis* grows in southern India, which lies in the tropics but north of the equator. *C. media* and *Bowenia serrulata* belong to the eastern part of Queensland, Australia, not extending far south of the Tropic of Capricorn; *Stangeria paradoxa*, in South Africa, extends from the tropics into the south temperate zone. All the species of *Encephalartos* studied belong to subtropical South Africa. *E. caffer* and *E. Altensteinii* are both found in open dry places, the former being abundant at Van Staadens, thirty miles inland from Port Elizabeth; and the latter at East London. Xerophytic structures are extensively developed in the leaflets of both species. *E. villosus* and *E. Hildebrantii*, although growing nearer the tropics than these species, are somewhat shaded. Their leaflets are decidedly less xerophytic in structure than those of *E. caffer* and *E. Altensteinii*. *Bowenia serrulata*, however, has not a development of xerophytic structures in proportion to the conditions to which it is exposed. It, with *Cycas media*, is found in the open *Eucalyptus* forests of Queensland, which afford little shade. The leaflet is broad and thin. It is well cutinized, and its glossy surface is undoubtedly very effective in reflecting the rays of light. Aside from the cutin and heavy-walled epidermis, structures protective against transpiration are wanting. There is no differentiated hypodermis or palisade, but the cells of the mesophyll are compactly arranged. Stomata are present on both surfaces. According to NESTLER there are thirty-five stomata to the square millimeter on the under surface, and fourteen to the square millimeter on the upper surface near the base of the leaflet. This is neither the largest nor the smallest number reported in the species examined by NESTLER. On the lower epidermis *Dioon edule* has eighty-one and *Ceratozamia brevifrons* twenty-four stomata to the square millimeter. The development of xerophytic structures in *Bowenia serrulata* seems hardly sufficient to account for the fact that the cut leaves may be exposed to the tropical sun for days and still remain fresh and green.

Among the Cycadaceae of the western hemisphere, with the exception of the two species of *Dioon*, structural development and environmental factors agree fairly well. *D. edule* is found at Chavarillo near Jalapa, Mexico, and *D. spinulosum* is abundant

at Tierra Blanca, seventy miles south of Vera Cruz. The former species grows in the open exposed to the blazing sun, and the latter in hot damp places well shaded by larger trees. During the wet season both species receive plenty of rain, and during the dry season there may be an occasional rainfall in the *D. spinulosum* locality. The general habit of the plants is quite in accordance with the conditions of their habitat. *D. edule* has a much smaller leaflet than *D. spinulosum* and a trunk only five feet high, while *D. spinulosum* commonly reaches a height of ten to thirty feet. The anatomical structure of the leaflets is not what would be expected from a consideration of the habitats. The cross-sections of the leaflets of the two species, as already stated in the description, are almost identical.

The genera of Cycads have so many striking characteristics in common that there can be no doubt of their close relationship, and in some of the genera the resemblance between species is so close that one is tempted to point out the parent species and its derivatives. In considering the structures in relation to environment, it must be kept in mind that leaflets, as we find them, are the product of two factors, environment and heredity, with heredity tending to keep the structures stable, and environment tending to modify them so as to make them most suited to their surroundings.

A quantitative study of the differences between plants, leaves, and leaflets growing in the field and in the greenhouse would be worth while if material were available.

Summary

1. A key based on topographical characters of leaflets is workable for genera.

2. The leaflets of the different species vary in color from bright green to dark green or dull gray-green; the shape and texture of the leaflets show an equal amount of variation.

3. A thick-walled, cutinized epidermis is characteristic of all the species examined; and a hypodermis is differentiated to some extent in all the species studied except *Bowenia*.

4. A well differentiated upper palisade is present in *Cycas*, *Dioon*, *Encephalartos*, *Ceratozamia*, *Macrozamia*, and *Zamia fur-*

furacea. Other species of *Zamia* show a more or less palisade region, but the cells are scarcely elongated or regular enough to constitute a true palisade.

5. The spongy mesophyll in most of the species examined is composed of transversely elongated cells. In nine of the species the cells are yoke-shaped.

6. In all of the species studied, thick-walled cells are found around the vascular bundles or scattered through the mesophyll.

7. Mucilage canals are present in *Macrozamia*, *Dioon*, *Stangeria*, and some species of *Encephalartos*.

8. In all of the species considered, except *Bowenia serrulata*, crystals of calcium oxalate are found.

9. Stomata were found on the upper as well as on the lower surfaces of the leaflets of *Macrozamia Moorei* and *Bowenia serrulata*. In the other species examined they were found on the lower surface only.

10. In studying the character of the leaflets two factors, heredity and environment, must be taken into consideration. The favorable environmental factors in greenhouse cultivation have perhaps modified the xerophytic structure of the leaflets studied.

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COLLEGE OF ST. SCHOLASTICA
DULUTH, MINN.

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EXPLANATION OF PLATES XV, XVI

Abbreviations: *c*, cutin; *e*, epidermis; *s*, stoma; *h*, hypodermis; *bf*, bast fiber; *p*, palisade tissue; *p'*, palisade-like tissue; *x*, xylem; *ph*, phloem; *scl*, sclerenchyma; *mc*, mucilage canal; *c'*, calcium oxalate crystal; *sv*, side vein.

FIG. 1.—*Microcycas calocoma:* diagram of portion of transverse section of pinnule showing distribution of bundles; ×25.

FIG. 2.—Same: showing detail of structure; ×120.

FIG. 3.—*Bowenia serrulata:* diagram of portion of transverse section of pinnule showing distribution of vascular bundles and bast fibers; ×55.

FIG. 4.—*Stangeria paradoxa:* diagram of portion of transverse section of leaflet showing side veins and distribution of vascular bundles in midrib; ×12.

FIG. 5.—*Cycas circinalis:* diagram of portion of transverse section of pinnule showing vascular bundle in midrib and transfusion tissue; ×12.

FIG. 6.—*Ceratozamia longifolia:* diagram of portion of transverse section of pinnule showing palisade tissue and bast fibers scattered through mesophyll; ×25.

FIG. 7.—*Macrozamia Moorei:* drawing of part of transverse section of pinnule showing stomata on both surfaces of leaflet; ×120.

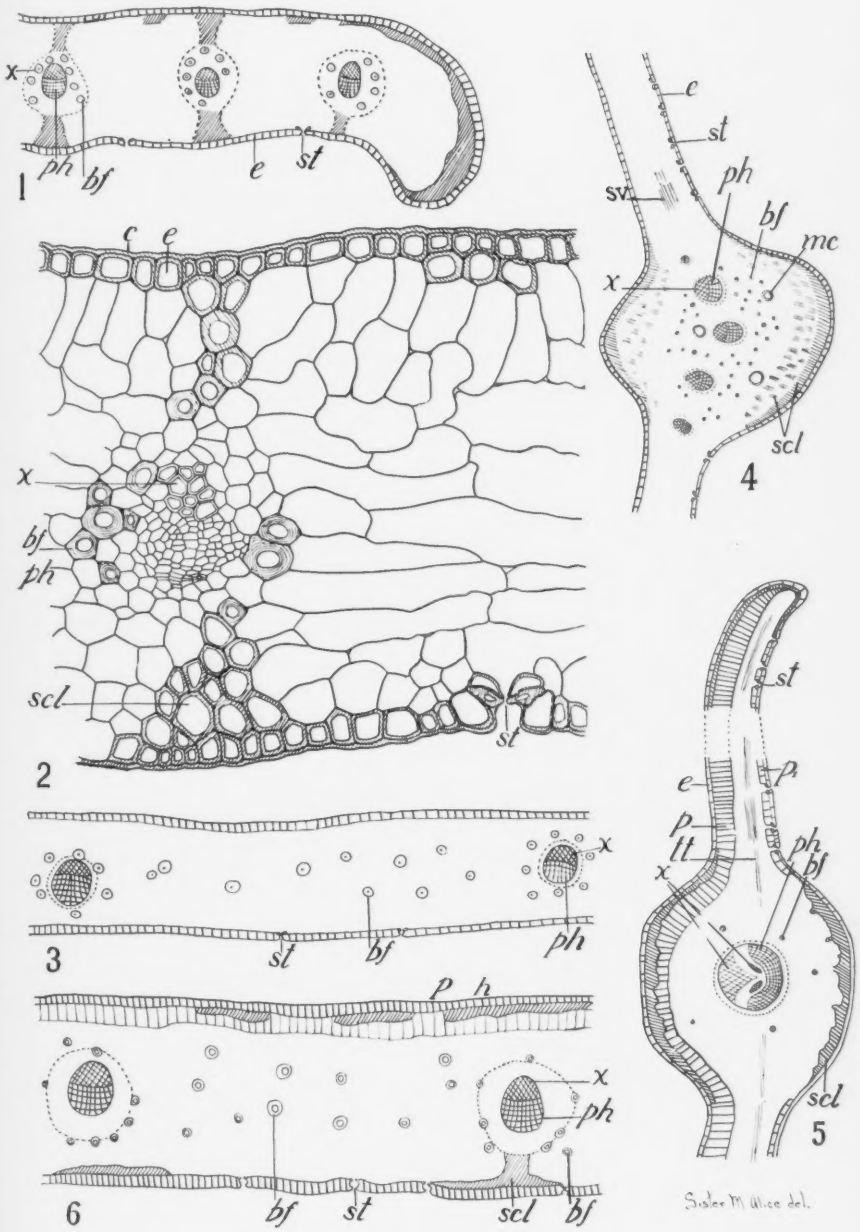
FIG. 8.—Same: diagram showing distribution of vascular bundles and mucilage canals; ×25.

FIG. 9.—*Dioon spinulosum:* diagram of portion of transverse section of leaflet showing mucilage canals above vascular bundles; ×45.

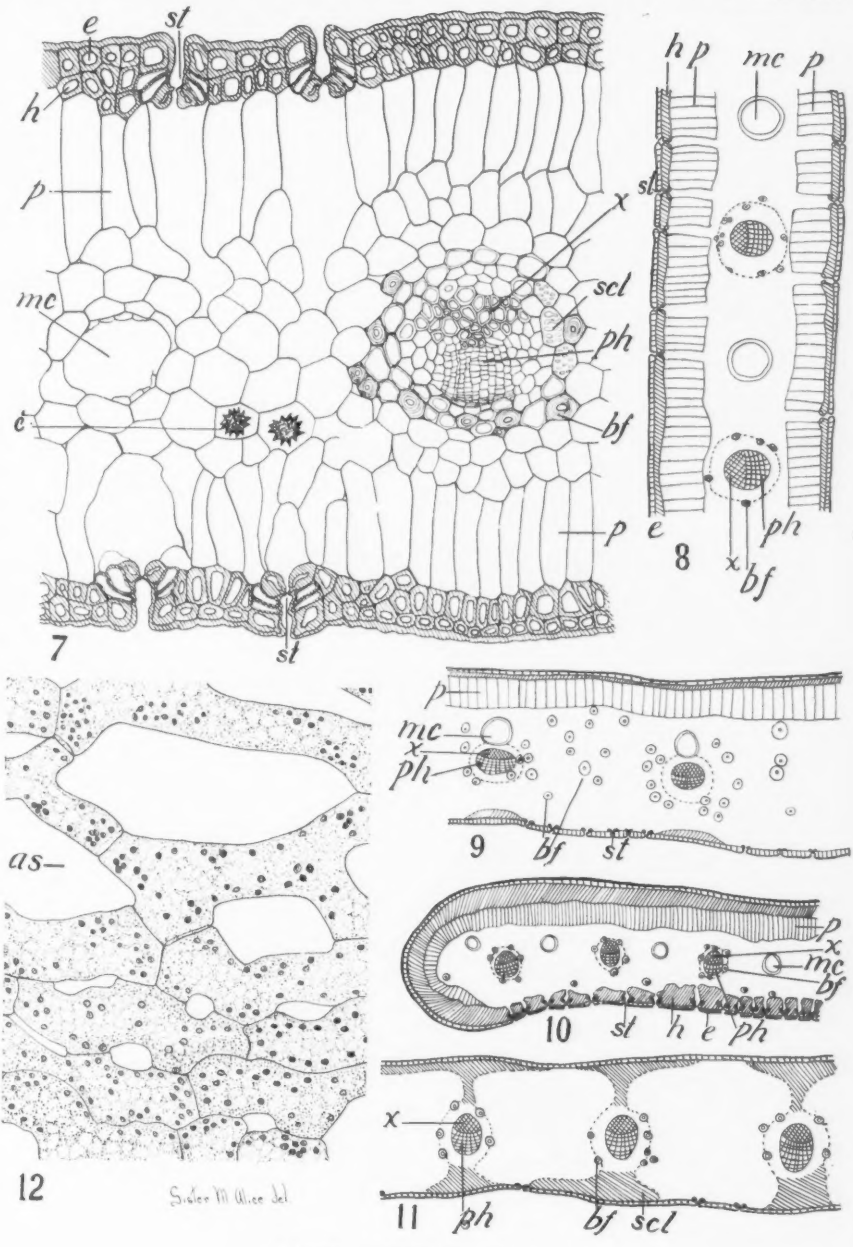
FIG. 10.—*Encephalartos Altensteinii:* diagram of part of transverse section of leaflet showing extensive development of hypodermis; ×12.

FIG. 11.—*Zamia Ottonis:* diagram of portion of leaflet showing bands of sclerenchyma above and below vascular bundles; ×25.

FIG. 12.—*Zamia furfuracea:* part of spongy tissue showing peculiar bridling of cells and large air spaces; ×120.



LAMB on CYCADACEAE



LAMB on CYCADACEAE

COMPARATIVE ANATOMY OF LEAF OF CYCADS, WITH REFERENCE TO CYCADOFILICALES[1]

JAMES P. POOLE

(WITH PLATES XVII–XIX)

In 1887 WILLIAMSON (**10**) recognized "the presence in the Carboniferous flora of plants combining the characters of ferns and Cycads." Later observers, in describing the anatomy of *Lyginodendron*, *Heterangium*, and *Medullosa*, discovered in them an anatomical structure distinctly intermediate between the two groups. In 1889 POTONIÉ (**7**) named the group Cycadofilicales, basing it on anatomical characters and indicating by its name its intermediate relationship. The group continued to be associated with the Pteridophytes, until in 1903 OLIVER and SCOTT (**6**) announced that certain seeds belonged to the fronds of *Lyginodendron Oldhamium*, thus definitely removing the Cycadofilicales from the Pteridophyta. Since that time other seed-bearing fronds have been discovered, until now all of the important genera of the Cycadofilicales are known to have borne seeds. The group name Pteridospermeae, to include those Cycadofilices that bear seeds, was proposed by OLIVER and SCOTT. Later investigation indicated that this group was an extensive one, and in 1909 COULTER (**2**) proposed that since it seemed impossible to recognize a group of Pteridospermeae as distinct from the Cycadofilices, and since there is a growing impression that the two groups are co-extensive, it would seem proper to retain the older name for the group, giving it the termination that would make it conform to the other groups of Gymnosperms with which it seems properly to coordinate, thus designating it as the Cycadofilicales. The two names Pteridospermeae and Cycadofilicales are now generally used synonymously.

In general there are two views as to the origin of the Cycadean type of vascular anatomy. One, advanced by SCOTT (**8**), would derive the type characteristic of the Cycadophyta from that possessed by Pteridosperms of the Lyginopteridean alliance. The

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

other view, that the Cycadean anatomy has been derived from the protostelic Medulloseae, was first advanced by POTONIÉ in 1889, but was independently stated and extended by WORSDELL (11) some years later. The more recent description of *Sutcliffia* (a genus originally described by SCOTT) by Miss DE FRAINE (4) is concluded by a third hypothesis as to the origin of the Cycadean type of anatomy, advanced by its author. In reality, however, this is a modification of the view advanced by POTONIÉ and WORSDELL, bringing it more closely into contact with that of SCOTT. From this view, *Sutcliffia* is considered as a primitive type from which two divergent lines of evolution may have arisen. The first proceeded through fairly simple types, like *Medullosa anglica*, to complex types with numerous steles, and there ended blindly, evidently because of insufficient plasticity for further evolution to take place. The second line remained protostelic throughout, the original vascular system becoming modified and giving rise to the Cycadean type. These hypotheses have been described in considerable detail by Miss BANCROFT (1).

In a concluding chapter, SCOTT (8) modifies his hypothesis to a certain extent, as follows:

> On the other hand, the structure of the petiole and the organization of the leaves generally are very similar in Medulloseae and Cycadaceae, and the resemblance extends to the Bennettiteae of the Mesozoic. On the whole of the evidence available it appears likely that some at least of the Cycadophyta may have been derived from plants resembling the Medulloseae in certain characters, though probably with monostelic rather than polystelic structure of the stem.

In order to render available additional evidence as to the affinity between the Cycadofilicales and the Cycadophyta, this investigation was undertaken. Incidentally, it has also brought out certain evidence which would seem to be applicable to the question as to the relative primitiveness of the Cycadeae and the Zamieae in the extant group.

An investigation of the anatomical structure of the petioles of the Cycads indicates certain striking resemblances to the same organs in the Pteridosperms. These similarities have been pointed out by earlier investigators, but never extensively studied. Without exception, the petioles of the Cycadean genera are traversed by

numerous vascular bundles. In this character they resemble the Medulloseae, in which a similar vascular system occurs in the petioles of all of the genera which have thus far been described. On the other hand, in the Lyginodendreae, the petioles are in all cases traversed either by two distinct vascular strands, or a single bundle resulting from fusion, a situation which does not occur in any of the genera of the Cycadophyta.

Further and even more striking evidence of the affinity between the two groups is found in the structure of the cortical armor, which is present in the stem and the leaf of the Cycadofilicales and persists in the petiole alone of the Cycadophyta. In the arrangement of the fibers which compose this cortical armor, certain features would seem to be of significance in relation to the phylogeny of the more modern group. Figs. 1 and 2 show the structure of the cortex in the stem of *Lyginodendron Oldhamium* from the transverse and tangential aspects. In the former the fibers are seen as plates of dark cells running through the cortex, extending radially from the region of the phloem to the epidermis, which in this case was removed previous to the petrifaction of the stem. In the case of the stem of this species, these fibrous strands anastomose, as is indicated by the fact that in places they appear to fuse, as seen in the transverse section, and becomes very evident on examination of the tangential aspect of a similar cortex, which shows the armor as a fibrous reticulum with vertically elongated meshes.

The situation in the rachis of *Lyginodendron Oldhamium* is shown in figs. 3–5. Fig. 3 shows that this organ is invested by a similar armor of platelike strands. From an examination of the outer region of the section more highly magnified (fig. 4), it is apparent that there is less of a tendency toward a fusion between the bands than in the stem cortex, a fact shown more clearly in the tangential section of the same structure (fig. 5). It would seem that these strands of cortical armor are common to both the stem and the rachis of the leaf in this ancient genus, but, whereas they are anastomosing in the stem, they extend through the rachis more nearly as separate vertical fibrous bundles.

Similar cortical structures are found in the Medullosean group. Fig. 6 shows the cortical zone in the stem of *Medullosa anglica*, and in fig. 7 the same region from the stem of *Sutcliffia* is shown.

In both of these genera it is evident, from the lack of any tendency toward a fusion of the plates, that the situation resembles more closely that seen in the *Lyginodendron* rachis, in which the bands run parallel, than that which is typical of the stem of the latter genus, in which the strands form a network. That similar structures persisted into the Cordaitean group is evident from an examination of the outer stem cortex of such a genus as *Mesoxylon*, in which the fibrous plates are evident as definite structures. This is of interest here only inasmuch as it indicates that the possession of the plate-like cortical armor was a character so firmly fixed upon the Cycadofilicalean group, that it persisted for some time in all the groups which branched from it. The hypodermal structures in the leaf of *Pinus* and some of the related genera seem quite clearly to be remnants of this character as it came down through the Cordaitean affinity.

In all of the Cycadophyta there is a very evident cortical armor in the petioles, and in some of them the resemblance of this structure to the homologous one in the Cycadofilicales is very striking. Of all the genera, this seems to be best exemplified in *Encephalartos*. Fig. 8 shows a transverse section of the petiole of *E. horridus*. Throughout the central portion this organ is traversed by numerous vascular bundles, a situation previously referred to as a characteristic feature of the Cycadean petioles, and pointed out as being a constant character in the petioles in the genera of Medullosean affinity. The same situation is evident in all the petioles later described, but will not again be alluded to in the descriptions. More pertinent to this discussion is the character of the cortex. Just beneath the epidermis are evident plates of fibrous cells which make up the cortical armor. An examination of these structures, as seen more highly magnified in fig. 9, and a comparison of this illustration with those which show the homologous structures in the Medullosean group (figs. 6, 7), will bring out the striking similarity of the two types. The same resemblance is very marked in *E. Altensteinii* (fig. 11). That these fibrous plates do not form a reticulum, but run in parallel strands, is evident from an examination of fig. 10, which shows their tangential aspect in *E. horridus*. A similar view of the strands in *E. Altensteinii* shows exactly the same situation.

In either of these sections the strands of thick-walled fibers may be seen alternating with plates of short parenchyma cells.

In *Dioon* the armor of the petioles is arranged in definite plates (fig. 12), but the resemblance to the Medullosean condition is not quite so marked as in *Encephalartos*. Seen in tangential aspect, as in fig. 13, it is similar in appearance to that of the genus previously described, varying only in the fact that perhaps the strands are not quite so widely separated. In *Zamia* the arrangement of the cortical fibers into plates is less evident, as seen in fig. 14, which shows a transverse section of the petiole of *Z. furfuracea*. In *Ceratozamia* the arrangement of these cells into groups can be distinguished only with difficulty, as is shown in the transverse section of *C. Kuesteriana* (fig. 15).

In the Cycadeae the cortical armor is also present, but the fibrous cells are arranged differently, and the relationship of the structure to that of the ancient group is less evident. Fig. 16, a portion of a transverse section of the outer cortex in the petiole of *Cycas revoluta*, shows the nature of the armor in this species. It is apparent that here the platelike strands are not formed, but the armor is a continuous one, composed of fibrous and parenchyma cells intermingled. That there is no evidence of the formation of platelike strands is still more apparent from an examination of a tangential section through the same region of the petiole (fig. 17). Here the fiber cells and the rows of parenchyma cells are clearly seen to alternate with one another, in direct contrast with the condition shown in *Encephalartos* and *Dioon* (figs. 8–13).

A similar arrangement of the fibrous cells of the cortex was found in all the species of *Cycas* examined, and would seem to be quite typical of the genus. The great similarity between these species is further emphasized by the fact that an examination of a small area of the outer cortex of the petioles of *C. media*, *C. celebica*, and *C. circinalis* indicates that it is impossible to distinguish one species from the other, or from *C. revoluta*. Fig. 18, showing a similar view of the cortical armor in *C. media*, is presented for comparison with *C. revoluta* (fig. 16). Tangential sections of the outer region of the petioles of *C. circinalis* and *C. Riuminiana* show that the absence of any tendency for an arrangement in plates

is just as evident as in *C. revoluta*. In *Macrozamia* and *Bowenia* the situation is very similar to that described for *Zamia furfuracea*. Material of the petiole of *Stangeria* was not available for the purpose of this examination.

Discussion

In the description of the cortical armor in the stem of *Lyginodendron*, it was pointed out that the strands formed a reticulum. SCOTT confirms this observation, and speaks of the petiole as being invested with a similar type of armor. In describing the cortex of the stem of *Heterangium* he says: "The fibrous strands take a straight vertical course, and only anastomose with one another at long intervals." In regard to its cortical ribbing, he also describes the petiole of this genus as resembling its stem. In his description of the petiole of *Medullosa* he speaks of a broad hypodermal zone consisting of numerous vertical strands of fibers interspersed with parenchyma. In regard to the petiole of *Sutcliffia*, SCOTT (**9**) says: "The leaf bases and detached petioles were of similar structure (to the stem), the hypoderma having radially arranged sclerotic bands as in *Myeloxylon*." This latter name was assigned to the fossil which later proved to be the petiole of *Medullosa*. Apparently, in the Medulloseae, as well as in the Lyginodendreae, the type of cortical armor in the petioles resembled that of the stems in each of the genera.

The presence of these bands of cortical armor in the petioles of both the Cycadofilicales and the Cycadophyta would seem to furnish additional anatomical evidence of the affinity between these two groups. The fact that the greatest resemblance is between certain of the Zamieae and the Medulloseae, namely, those forms having the cortical armor in vertical platelike bands, would seem to allow the interpretation that the two groups were in closer contact at those points than at any other. Arranging the groups in a linear series, we have at one end the Lyginodendreae with the cortical fibers grouped into plates, but with these latter structures reticulating. Next come the Medulloseae with the cortical armor in vertical parallel strands, and these are followed by the Zamieae with a similar structure, the groups of cells being very distinct in certain genera, but in others approaching the con-

dition typical of the Cycadeae, which might be placed at the other extreme of the series, where the cortical armor is of the continuous type.

Such an interpretation of the comparative anatomy of the two groups would seem not only to add further evidence as to the Medullosean affinity of the Cycadophyta, but also to indicate that the Zamieae are nearer the base of the Cycadean line of descent than are the Cycadeae. In order to throw further light on this question as to the relative primitiveness of the Zamieae and the Cycadeae, a comparison of the structure of the laminae of the leaves in the Cycadophyta and the Cycadofilicales was undertaken.

SCOTT (8) describes the leaves of *Lyginodendron* (*Sphenopteris Honinghausi*) as being deeply lobed, with veins diverging from one another at an acute angle. In describing the leaf of *Heteangium*, he says: "The evidence available from the petrified specimens leads to the conclusion that the foliage was of the same type, as regards both anatomy and general Sphenopterid form, as that of *Lyginodendron Oldhamium*."

The leaf of *Medullosa* is now known to be the type which was first described as *Neuropteris*, and is described by SCOTT (8) as having oblong leaflets, each of which possesses a median vein, breaking up into smaller dichotomous nervules near the end, with similar nervules given off from the sides of the median nerve throughout its length. He also speaks of the leaflets of *Medullosa anglica* as having lateral bundles which traverse the lamina in an oblique direction. The investigations of WIELAND on the Bennettiteae are also quoted by SCOTT as indicating in the anatomical structure of their leaves a close agreement with those of recent Cycads of the suborder Zamieae. Thus it is apparent that all the ancient groups to which the Cycads show an affinity were characterized by many veined leaflets, with perhaps a tendency among some of the Medulloseae toward the formation of a midvein.

In the Cycadophyta, all of the Zamieae are characterized by the possession of numerous more or less parallel veins. In *Stangeria* there is a midvein, but it is accompanied by an abundance of lateral veins which diverge from it. On the other hand, all of the Cycadeae have one midvein with a complete lack of any lateral

bundles. This comparison, therefore, of the vascular anatomy of the leaves of the extant group with that of the leaves of the group from which it has apparently originated, shows that the greater similarity is between the ancient forms and the Zamieae rather than the Cycadeae. To the extent that greater similarity in structure indicates closer relationship, this evidence would seem to furnish additional indication of the Zamieae being more closely related to the Cycadofilicales than the Cycadeae, and hence the more primitive of these two suborders.

The assignment of *Cycas* to a primitive position in the Cycadaean series has been based largely on the fact that the ovuliferous structures are borne on sporophylls that are attached directly to the vegetative axis, rather than in a strobilus, as in the Zamieae. It is true that this condition resembles that in the Cycadofilicales, as stated by COULTER and CHAMBERLAIN (3), but in this connection it might be pointed out that with the lack of any certain fossil evidence of the nature of the reproductive structures in the extinct Cycadophyta, such a feature cannot well be considered primitive beyond question of a doubt. Throughout the whole plant kingdom so many structures, once considered as indicating a primitive position, upon investigation of the transitional stages in their development have proved to illustrate a condition of reduction instead, that it might be stated as a general principle that any organ, although simple in structure, should not definitely be assigned to either a primitive or a reduced category until both the ontogenetic and the phylogenetic histories of the organ are satisfactorily investigated. Since such information is as yet unavailable in the case of the reproductive structures of *Cycas*, these organs might equally as well be considered to be reduced structures as primitive ones.

In this connection it may be pointed out that, throughout the modern Gymnosperms, it is the staminate cone which more persistently retains the primitive type of organization than does the ovulate. An example of this situation is seen in *Taxus*, in which the microsporangial structures are borne in a strobilus similar to the staminate cones of other Coniferales, but the seed cone is in the reduced condition peculiar to that genus. JEFFREY (5) states:

> The conservative tendencies of microsporangial structures as contrasted with those of the megasporangium or seed, which is considerably more rapidly influenced by the course of evolution, constitute a valuable situation from the standpoint of comparative anatomy.

This principle is well supported by evidence drawn from several groups, and it would seem that it might well be applicable to the case in point.

Conclusions

The peculiar structures of the petiolar cortex herein described furnish additional evidence of the descent of the Cycadales from the Cycadofilicales. The further fact that the cortical armor of the petioles of the Cycadophyta shows a closer resemblance to homologous structures in the Medulloseae than in the Lyginodendreae would seem to indicate that the more modern group has a closer affinity with the former than with the latter alliance. This evidence is further strengthened by the greater similarity in the system of vascular supply in the Medullosean and the Cycadean petioles as compared with the same situation in the Lyginodendreae.

Following out the same line of reasoning, the evidence presented by the structural features in question would seem to imply that the Zamieae are nearer the base of the Cycadean line of descent than are the Cycadeae. In this case also the evidence adduced from the cortical structures is further supported by the study of the vascular supply system of the pinnae of the leaves. It has been pointed out previously that in the possession of numerous veins the leaves of the Zamieae resemble more closely the Cycadofilicales than do the Cycadeae with a single vein. *Lyginodendron* leaves have been described as possessing several veins. The descriptions of the Medullosean leaves mention a midvein, but also several lateral veins, and in the Bennettiteae the leaves have been described as possessing a vascular supply very similar to that of the Zamieae. Apparently the evidence furnished by the ancient fossils points clearly to the fact that the possession of numerous veins was the primitive condition. This interpretation of the facts is still further borne out by an examination of the literature in regard to the fossil Cycadophyta. The only form genus described as showing a tendency toward a reduction in the number of veins is *Pseudocycas*,

established by NATHORST, and found in the lower Cretaceous horizon, whereas the other fossil genera, all characterized by the possession of numerous veins, ranged from the Triassic to the Lower Cretaceous. There is an indication even that some of these latter forms existed in the Permian, but they were clearly and abundantly present in the Jurassic. The natural conclusion would be that the type of leaf with numerous veins existed previous to the form with a single midrib, and that the latter type has been developed through a process of reduction.

It is recognized that to thus assign to the Zamieae a more primitive position than the Cycadeae in the Cycadean line of descent, is contrary to the generally accepted opinion as to the relative phylogenetic positions of these two groups. It has been shown, however, that the structures upon which the more commonly accepted view is largely based are susceptible of more than one interpretation, and the evidence which they furnish might be adduced to signify a reduction in a more recent group as well as a simplicity in a primitive type. This fact, considered in conjunction with the structure of the fossil leaves, as here presented, would seem to indicate that these commonly accepted views are none too soundly established and might well be examined critically.

In consideration of the amount of evidence available, it would undoubtedly be somewhat dogmatic to state definitely that the Zamieae are the more ancient of the two suborders, but it does seem quite indisputable that the balance of the evidence rendered available by this investigation inclines in that direction. Furthermore, this view derives additional support from certain structural features of the pinnae of the two groups, which will be described in a later paper.

Summary

1. The similarity in the structure of the cortical armor of the petioles in the Cycadophyta and the Cycadofilicales furnishes additional indication of the affinity between these groups.

2. The structural features of the petiole in the Cycadophyta resemble the Medulloseae rather than the Lyginodendreae, thus giving evidence of a closer connection with the former than the latter group.

3. The structural features of the petiole in the Zamieae resemble the Medulloseae more closely than do the homologous structures in the Cycadeae, a situation which would indicate a more primitive position for the former suborder.

4. In the possession of many veined leaves, the Zamieae resemble more closely the ancient group than do the Cycadeae, and therefore would seem to be the more primitive of the two suborders.

5. The paleobotanical evidence at present available indicates that the many veined type of leaf was of earlier occurrence among the Cycadophyta than the type with a reduced number of veins.

6. The more simple structure of the female reproductive axis in the Cycadeae has been adduced as an argument in favor of the more primitive position of this suborder, but there is no sound evidence which would indicate that this structure is a primitive one rather than a reduction. This situation renders such evidence as to the primitiveness of the Cycadeae of doubtful value.

HARVARD UNIVERSITY
Cambridge, Mass.

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EXPLANATION OF PLATES XVII–XIX

PLATE XVII

FIG. 1.—Transverse section of cortex of stem of *Lyginodendron Oldhamium*, moderately magnified to show plates of cortical armor.

FIG. 2.—Tangential section of cortex of stem of *Lyginodendron Oldhamium*, moderately magnified to show armor as network of fibrous strands.

FIG. 3.—Transverse section of rachis of leaf of *Lyginodendron Oldhamium*, moderately magnified to show hypodermal bands of sclerotic cells and vascular tissue.

FIG. 4.—Part of fig. 3, more highly magnified, showing hypodermal bands in more detail.

FIG. 5.—Longitudinal section of part of rachis of *Lyginodendron Oldhamium*, moderately magnified to show hypodermal bands in tangential aspect.

FIG. 6.—Transverse section of cortex of stem of *Medullosa anglica*, moderately magnified to show plates of cortical armor.

PLATE XVIII

FIG. 7.—Transverse section of cortex of stem of *Sutcliffia insignis*, moderately magnified to show plates of cortical armor.

FIG. 8.—Transverse section of petiole of *Encephalartos horridus*, slightly magnified to show numerous vascular bundles and cortical armor.

FIG. 9.—Part of fig. 8, more highly magnified to show platelike formation of cortical armor.

FIG. 10.—Tangential section of cortex of petiole of *Encephalartos horridus*, moderately magnified to show longitudinal aspect of strands of cortical armor.

FIG. 11.—Transverse section of cortex of petiole of *Encephalartos Altensteinii*, moderately magnified to show platelike formation of cortical armor.

FIG. 12.—Transverse section of cortex of petiole of *Dioon spinulosum*, moderately magnified to show platelike formation of cortical armor.

PLATE XIX

FIG. 13.—Longitudinal section showing tangential aspect of tissue shown in fig. 12.

FIG. 14.—Transverse section of cortex of petiole of *Zamia furfuracea*, moderately magnified to show arrangement of sclerotic cells of cortical armor.

FIG. 15.—Transverse section of cortex of petiole of *Ceratozamia Kuesteriana*, moderately magnified to show arrangement of sclerotic cells of cortical armor.

FIG. 16.—Transverse section of cortex of petiole of *Cycas revoluta*, moderately magnified to show continuous type of cortical armor.

FIG. 17.—Longitudinal section showing tangential aspect of cortical tissue illustrated in fig. 16.

FIG. 18.—Transverse section of cortex of petiole of *Cycas media*, moderately magnified to show continuous nature of cortical armor.

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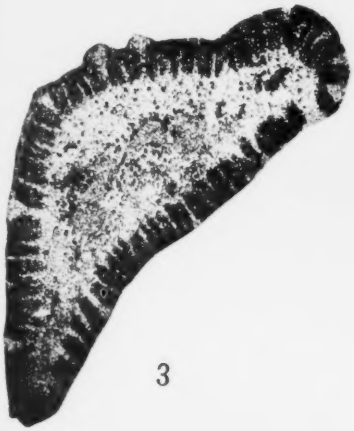

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POOLE on CYCADS

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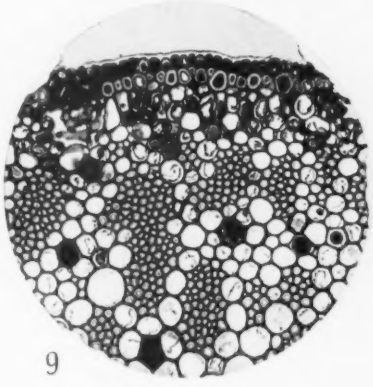

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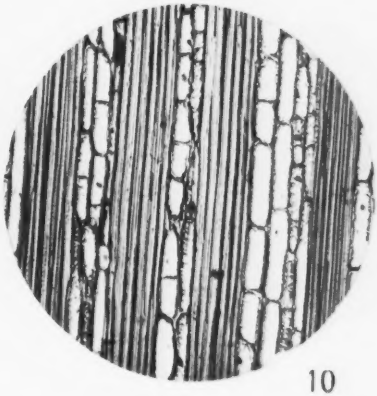

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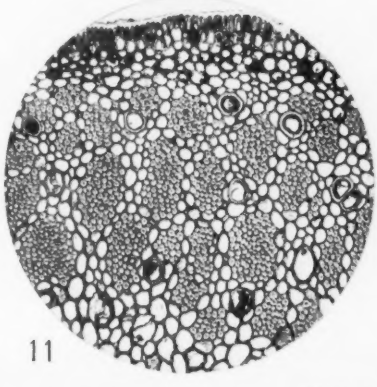

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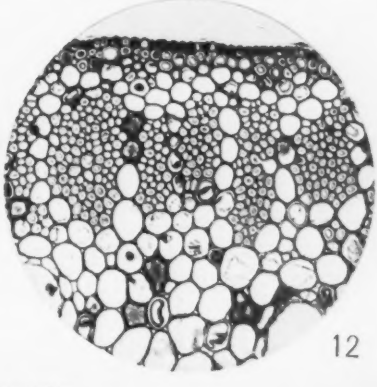

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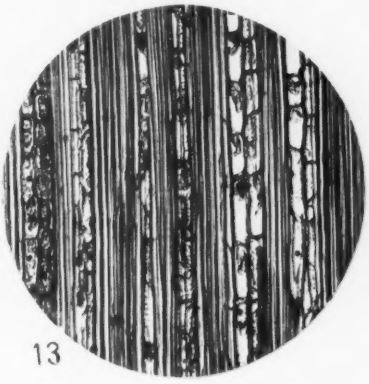
POOLE on CYCADS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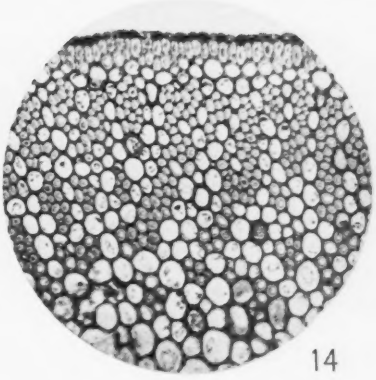
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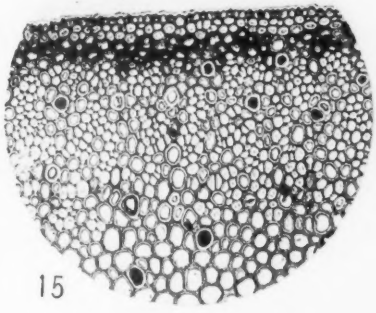
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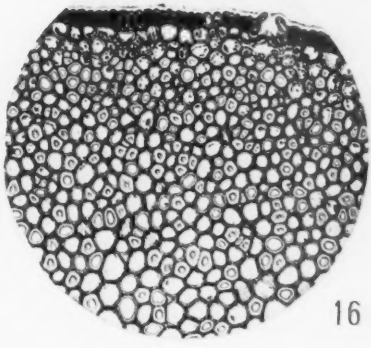
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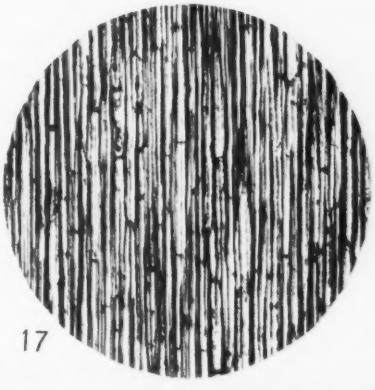
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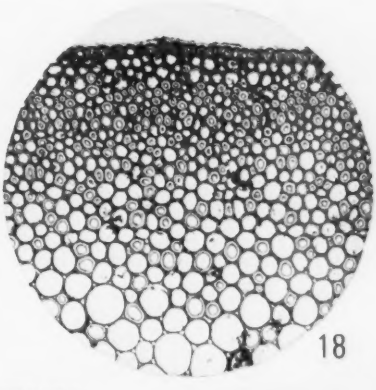
18

POOLE on CYCADS

BRIEFER ARTICLES

A SUPPOSED PALEOZOIC ANGIOSPERM

The paper on *A Paleozoic angiosperm from an American coal ball* by J. H. HOSKINS in a recent number of the BOTANICAL GAZETTE (**75**: 390–399. *pl. 17. figs. 7.* 1923) is one which will naturally create some excitement among botanists interested in the evolution and antiquity of the angiosperms, and probably all botanists are interested in the solution of this "abominable mystery." The title suggests at least the discovery of some genuine evidence of the occurrence of an angiosperm among the relics of a Paleozoic flora. The fossil described by HOSKINS is, I am convinced, a specimen of the well known Medullosean petiole *Myeloxylon*. Material identical with that from Illinois is common in European countries in Upper Carboniferous rocks, and the late Professor PENHALLOW described examples from Upper Carboniferous strata in Kansas. The photographs reproduced in pl. XVII are typical illustrations of *Myeloxylon*. Recent discoveries by HAMSHAW THOMAS demonstrate the existence of a type of fruit with the essential angiospermous characters in the Jurassic beds of Yorkshire, and it may well be that even older representatives of the present dominant class will be found; but it may confidently be asserted that as yet we have no evidence whatever in support of the existence of angiosperms in pre-Jurassic days. HOSKINS states that his description "differs in all fundamental respects from that of any known cycadean petiole type such as *Myeloxylon*." One would like to know where the difference lies. In the drawing of a vascular bundle on p. 396 the position of the protoxylem is not indicated. In *Myeloxylon* it is on the side of the xylem next the phloem. If the Illinois specimen shows the protoxylem in any other position the fact would be interesting. The institution of a new genus *Angiospermophyton*, I submit, is entirely without justification.—A. C. SEWARD, *Cambridge, England.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Cytology of Rosa.—This genus is bewildering to investigators no matter from what point of view they study it, but perhaps its most bewildering aspect is presented to the systematist. Its phenomenal polymorphism defies a classification that can be widely accepted, identification and relationships being baffling even to experienced rhodologists. Such a situation is inviting to the cytologist whose chromosome counts and reduction division studies may throw light on relationships otherwise obscure, and offer some explanation of the origin and causes of these extreme variations. TÄCKHOLM'S[1] work is an important contribution in this field. It is divided into two parts. The first is a classification of the genus based on its cytological investigation, together with complete accounts of reduction divisions in all the sections, but especially in the Caninae, where polymorphism is so extreme. The second part is an elaborate and detailed discussion of the possible mode of origin and of the development of the Caninae. This work has entailed a very great amount of careful and patient investigation whose record here provides a standard reference to the subject.

The fundamental chromosome number in *Rosa* is 7, and most regular true-breeding species have 7 pairs, although multiples of this number occur. There are also some hybrids with irregular numbers. In the Caninae section, however, there are 7 pairs and 14, 21, or 28 single chromosomes as well, as is demonstrated in the reduction division. Here the situation is always like that in F_1 hybrids from parents with different numbers of chromosomes, as in the classical case of *Drosera*. In both meiotic divisions the single chromosomes are late in splitting and in going to the poles, with the result that in the pollen mother cells they are seldom all included in the tetrad, and usually form nuclei of their own, thus producing from 8 to many abortive pollen grains or polynucleate non-functional spores. In the embryo sac mother cell a peculiar situation develops, as all but 7 chromosomes (one of each pair) are usually included in the egg cell, which thereby may be functional.

This F_1 type of meiosis is held by the author to indicate that all Canina forms are F_1 hybrids derived from one parent with 7 chromosome pairs, and the other with 14, 21, and 28 respectively, and that they remain so from generation to generation, because they produce seed asexually, probably by sporophyte (nucellar) budding. That this is the normal method of seed production is undisputed. No suitable roses have been found, however, with such high

[1] TÄCKHOLM GUNNAR, Zytologische Studien über die Gattung *Rosa*. Acta Horti Bergiani. **7**:97–381. *figs. 56.* 1922.

chromosome numbers as would be required of the original parents. The author's contention is that such roses must have existed before the ice age, and that the original crosses took place then. The glacial period exterminated the parents and left the F_1 hybrids, which still are true to type, due to non-sexual reproduction. Wide geographic distribution of the Caninae (Europe, Asia, and North Africa) is adduced as supporting this hypothesis. Polymorphism has arisen through "embryo mutation" which has given rise to very numerous constant forms, and through secondary hybridization, which produces for the most part ephemeral races with irregular chromosome numbers.

In spite of the very detailed discussion of this hypothesis, with copious references to similar cytological work done on this and other genera, the assumption that unknown parents, tetraploid, hexaploid, and octaploid (different from any such existing today) produced, by crossing with diploid forms in preglacial times, plants identical with the Caninae of today save for "embryo mutation," appears highly speculative, and, as well as accounting for the old, raises new questions such as why somatic mutations should be confined to the embryo. The rival theory of BLACKBURN and HARRISON[2] seems at least equally worthy of note. These authors hold that since some Canina pollen grains get only 7 chromosomes, and most egg cells have 7 plus the 14, 21, or 28 single chromosomes, fertilization here would restore the original number, and the segregation of the 7 chromosomes would provide for all the various races known, asexual reproduction causing these normally to breed true. They account for the high number of single chromosomes as the result of repeated back crosses, or of "mass homotypic divisions" such as they have observed where the two spindles coalesce. Such divisions have been observed by other workers also. This latter hypothesis might be tested by breeding, because reciprocal crosses should not be equivalent but rather strongly maternal, since the egg cell would contribute all but 7 of the F_1's chromosome complement. Whatever may be the verdict of the future as to the origin of these polymorph roses with their unusual chromosome equipment and irregular meiosis, there can be no doubt that TÄCKHOLM's work is a very important contribution to our knowledge of the subject, and will prove of unusual interest to all cytologists.—R. O. EARL.

Double fertilization in Ephedra.—The female flowers and fertilization in *Ephedra campylopoda* are described in a well illustrated paper by Miss Stephanie Herzfeld.[3] This species has two kinds of female flowers, one being strictly female and borne on strictly female plants, while the other has stamens below

[2] BLACKBURN, KATHLEEN B., and HARRISON, J. W. H., The status of the British rose forms as determined by their cytological behavior. Ann. Botany **35**:159–188. 1921.

[3] HERZFELD, STEPHANIE, *Ephedra campylopoda* Mey. Morphologie der weiblichen Blüthe and Befruchtungsvorgang. Denks. Akad. Wissen. Wien, Math.-Natur. Kl. **98**:243–268. *pls. 1–2.* 1921

the ovules. These modified female flowers resemble somewhat those found in *Gnetum Gnemon*. In both the normal and modified female flowers, a ring of tissue appears at the base of the ovule, and later becomes quite conspicuous. The integument makes its appearance between this ring of tissue and the nucellus, in the normal flower developing the long micropyle, but in the modified flower becoming more or less abortive and distorted. The ring is neither sporophyll, integument, nor perianth, but an "organ *sui generis*" and homologous with the fertile scales (Fruchtschuppen) of the Taxaceae.

The megaspore mother cell is clearly hypodermal, and gives rise to four megaspores, of which the lower one germinates. Any cell of the upper layer of the female gametophyte may be an archegonium initial, but usually only two or three develop into mature archegonia, with one to six the extreme range of numbers. No wall is formed between the ventral canal nucleus and the egg nucleus, and even traces of spindle fibers could not be identified. The microspore, at the time of shedding, has one prothallial cell, two "wall cells," a pollen tube nucleus, and an "antheridial cell" which later forms two male cells.

In several cases Miss HERZFELD found that one of the male nuclei fuses with the ventral canal nucleus, while the other fuses with the nucleus of the egg. This is described as "double fertilization." Referring to LAND'S work on *Ephedra trifurca*, in which he figures some small nuclei in the top of the fertilized egg, she sees another case of genuine double fertilization, and gives the same interpretation to some nuclei at the top of the fertilized egg of *Abies balsamea* as described by HUTCHINSON.

That one of the male nuclei in Coniferales may fuse with the ventral canal nucleus was established long ago. It is a curious fact that the first two cases of "fertilization" described for Coniferales turned out to be fusions of the ventral canal nucleus with the nucleus of the egg, and it was suggested soon after the second case appeared that, where the wall separating the ventral canal nucleus from the egg nucleus breaks down and the ventral canal nucleus becomes quite large, a male nucleus might fuse with this ventral canal nucleus.

While it is readily admitted that Miss HERZFELD has established that, in *Ephedra*, one male nucleus fuses with the ventral canal nucleus while the other fuses with the nucleus of the egg, and that similar fusions may occur occasionally in other gymnosperms, and also that a few nuclei may result from the fusion of a male nucleus with a ventral canal nucleus, this behavior should not be regarded as double fertilization like that of the angiosperms. There are no polar nuclei in *Ephedra*, and consequently there can be no triple fusion. Besides, in *Ephedra* both fusions take place within the egg itself, which precludes the formation of endosperm. It is surmised that *Gnetum* would be more likely to show a nearer approach to the double fertilization of the angiosperms. The x and $2x$ chromosome numbers are 6 and 12, an interesting variation from the prevailing 12 and 24 of the gymnosperms.—C. J. CHAMBERLAIN.

Morphology of Riccardia.—SHOWALTER[4] has contributed a paper on *Riccardia pinguis* dealing chiefly with the growth of the thallus and the development of the archegonium. He finds slight morphological variation, especially in the form of the antheridial branches, in plants growing under diverse field and cultural conditions. Growth of the thallus is by means of a "two-faced" apical cell (the common dolabrate type previously reported for this species), the early divisions following the scheme of LEITGEB. The general features of archegonium development are the same as in other Hepaticae, the most important difference reported relating to the time of formation of the ventral canal cell and egg with reference to the formation of the neck canal cells. Following the first division of the neck canal mother cell (primary neck canal cell), the central cell (primary ventral cell) divides to form the ventral canal cell and egg; later the two neck canal cells divide in acropetal succession, forming four (occasionally six). In most of the Hepaticae studied, the differentiation of the egg does not occur so early in the development. The author finds that the first walls in the embryo show an order of formation differing from that described by Miss CLAPP.[5]

FLORIN[6] confirms the results of previous investigations on the development of the archegonium of *Riccardia*, presenting some interesting anomalies, and adding certain details to the development of the sporophyte and sporogenesis. Like SHOWALTER, he finds that the egg and ventral canal cell are formed before the first two neck canal cells divide to form four. Developmental abnormalities, regarded by the author as "atavisms," include antheridium-like stages in the early development; unusual persistence of the ventral canal cell; occurrence in the venter of four morphologically equivalent nuclei derived by two successive divisions from the primary ventral cell; transitions between the archegonium and its hypothetical precursor, the plurilocular gametangium; extraordinary cases where the four axial cells have all assumed the form of eggs, thus actually constituting a plurilocular gametangium. The transition stages mentioned consist of an archegonium with a single egg and a binucleate ventral canal call, one with a single egg, and two rows of canal cells, and one having an egg and two ventral canal cells, one of the latter assuming the function of an egg.

In most cases difficulty was experienced in staining the ripe egg, but in a single favorable instance two distinct unipolar centrosome-like bodies were seen on opposite sides of the nucleus; these are called merely "Strahlungszentren."

[4] SHOWALTER, AMOS M., Studies in the morphology of *Riccardia pinguis*. Amer. Jour. Bot. **10**: 148–166. 1923.

[5] CLAPP, GRACE L., The life history of *Aneura pinguis*. BOT. GAZ. **54**: 177–193. 1912.

[6] FLORIN, RUDOLF, Zytologische Bryophytenstudien. III. Das Archegonium der *Riccardia pinguis* (L.) B. Gr. III. Die Entwicklungsgeschichte des Sporophyten und die Sporogenese bei *Riccardia pinguis* (L.) B. Gr. Archiv. Bot. **18**: 1–58. 1922.

In the spore mother cells no centrosomes were observed, and no multipolar spindles. Diakinesis is described and ten gemini reported as being formed. The author counted twenty chromosomes in the sterile cells of the sporophyte (seta) and ten in the gametophyte.—A. W. HAUPT.

Female gametophyte of Angiosperms.—RUTGERS[7] has made an extended analysis of the Angiosperm embryo sac, and has concluded that classifications based on the number of divisions between megaspore nucleus and egg, or on the number of nuclei in the fullgrown sac, are wholly artificial. He claims that the female gametophyte is not a morphological unit, but a complex. Any classification, therefore, must be based upon the following processes, which probably represent independent lines of development: chromatin reduction, megaspore formation, polarization, development of a micropylar group of nuclei, development of a chalazal group of nuclei. As a consequence, although the 8-nucleate sac seems to be the most characteristic type of the Angiosperm embryo sac, he was not able to throw any light on its origin.—J. M. C.

Anatomy of desert plants.—From the two Indian states of Jodhpur and Jaisalmer, SABNIS[8] has collected material for an anatomical study which has resulted in an accumulation of descriptive data that, while interesting, is poorly organized and leads to no very definite conclusions. The author is to be commended for the extensive and careful work, devoted to the accumulation of data that will become more valuable as time passes, for such an investigation will find most of its value in creating a volume of reference for the coming botanists of India.—GEO. D. FULLER.

Grassland invasion.—AITKEN[9] has given an account of the invasion of a South African grassland by trees, a stream valley being the basis of colonization. The principal grass is *Aristida junciformis*, and the commonest invading trees are *Combretum kraussii*, *Ficus capensis*, and *Cussonia spicata*. Fire seems to be one of the chief factors in checking such invasions. This type of veld with trees in its depressions is more mesophytic than the commoner thorn veld.—GEO. D. FULLER.

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[7] RUTGERS, F. L., The female gametophyte of Angiosperms. Reliquia Treubianae III. pp. 6–66. *pls. 6*. 1923.

[8] SABNIS, T. S., The physiological anatomy of the plants of the Indian desert. Jour. Ind. Bot. **1**:1–152. 1919.

[9] AITKEN, R. D., The plant succession in a type of Midland tree veld in Natal. So. African Jour. Sci. **18**:233–243. *figs. 5*. 1922.